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Analytical Method Development and Validation of Avelumab and Axitinib in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

Merkel cell carcinoma is treated with Avelumab, and kidney cancer is treated with Axitinib. Axitinib is used to treat advanced renal cell carcinoma in conjunction with avelumab (Bavencio) or pembrolizumab (Keytruda). So, in this session, we used RP-HPLC to establish an accurate, fast, cost-effective, and straightforward, reliable assay technique for the evaluation of Avelumab and Axitinib. Using acetonitrile and OPA (60:40 v/v) as a mobile phase, symmetry C18 column (150mmx4.6mm, 3.5 µ)with a flow rate of 1 ml/min, effective chromatographic separation was achieved, with the wavelength measured at 222 nm. The retention times (Rt) of Axitinibis were 3.158 minutes and Avelumab 4.429 minutes, respectively, when chromatography was conducted isocratically at room temperature with a 6-minute run time. The linearity analysis was conducted at 10 percent to 150 percent stages, and the regression coefficient of the two drugs was found to be 0.999. Axitinib and Avelumab are having precision results of 0.91 and 0.55, respectively. The recovery of the drugs was found to be 99-100 percent, which is within the appropriate range. RSD values were less than 2.0 percent indicating that this method is accurate and precise. According to ICH guidelines, the approach was justified. Degradation stress conditions in acidic, alkaline, peroxide, and thermal media were investigated. Under ideal conditions, the established method produced efficient, precise, and accurate results. As a result, it was clear that the proposed approach was ideal for routine pharmaceutical preparation review and quality control.

1. INTRODUCTION

Avelumab [1,2], also known as Bavencio, is a fully human monoclonal antibody used to treat Merkel cell carcinoma [3,4], urothelial carcinoma [5,6], and renal cell carcinoma [7,8]. Fatigue [9], musculoskeletal pain [10], diarrhea, nausea, infusion-related reactions, rash, reduced appetite, and limb swelling (peripheral edema) [11] are all popular side effects. Avelumab is a monoclonal antibody that targets the protein programmed death-ligand 1 (PD-L1) [12]. In January 2017, the European Medicines Agency (EMA) designated it as an orphan drug for the treatment of gastric cancer [13]. Immune-mediated adverse reactions (pneumonitis [14], hepatitis, colitis [15], adrenal insufficiency [16], hypo- and hyperthyroidism [17], diabetes mellitus [18], and nephritis) and life-threatening infusion reactions are the most frequent severe adverse reactions to avelumab.

Pfizer created **Axitinib** (AG013736; trade name Inlyta), a small molecule tyrosine kinase inhibitor [19]. It has been shown to prevent breast cancer [20] growth in animal (xenograft) models [21] and to show partial responses in clinical trials with renal cell carcinoma (RCC) [22] and many other tumor forms [23]. The US Food and Drug Administration approved it for RCC after it showed a small improvement in progression-free survival, though there have been reports of fatal side effects. The most common side effects are diarrhea, hypertension, fatigue, reduced appetite, nausea, dysphonia [24], hand-foot syndrome [25], weight loss, vomiting, asthenia, and constipation, which affect more than 20% of patients [26]. The aim of the study is to separate the pharma ingredients of Avelumab and Axitinib by using RP-HPLC.

Till today there are some UV, HPLC, and LCMS methods were reported in the literature for Axitinib, but no methods are developed in the individual analysis of Avelumab. Hence, we developed a method for the simultaneous quantification of Avelumab and Axitinib. The developed HPLC method was utilized for the estimation of the combined drugs in *In Vitro* method.

2. MATERIALS AND METHOD

Chemicals and materials: Merck (India) Ltd, Worli, Mumbai, India, provided HPLC grade acetonitrile (99.99% purity), Milli Q water, and Orthophosphoric acid. Both Axitinib (99.99% purity) and Avelumab (99.99% purity) APIs were obtained as reference standards from Zydus, Cadila, and Ahmadabad.

Equipment: Axitinib, and Avelumab, were isolated using a Waters alliance e2695 model HPLC with a PDA (photodiode array) detector and the chromatographic program Empower 2.0 [27].

Chromatographic Conditions: Using a symmetry C18 (150x4.6mm, 3.5) column, chromatographic separation was performed in an isocratic mode at room temperature. The mobile phase is an isocratic mixture of acetonitrile and OPA(60:40) with a flow rate of 1 ml/min with a detection wavelength of 225nm. The injection volume was 10 µl, with a 6-minute run time.

Preparation of standard solution: Working standards of 5 mg Axitinib and 20 mg Avelumab must be correctly weighed. These standards were put in a 100 mL volumetric flask, filled with 70 mL diluents, and sonicated for 10 minutes to dissolve the contents before being made up to the mark with the same diluents. Using the diluents, dilute 5 mL of the above solution to 50 mL.

Preparation of sample stock solution: In a 100 ml volumetric flask, measure correctly the 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 mL of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup up the mark with diluents. Using a 0.45 syringe filter, filter the solution. The Assay for the marketed formulation was shown in **Table no-1.01**.

Table-1.01 Assay for Marketed Formulation Calculation

S.NO	Formulation (Capsule)	Labeled amount (mg/Tab)	Amount Found	%Assay
1	Avelumab	20mg/ml	19.2mg	99.6%
2	Axitinib	5mg/ml	4.975mg	99.5%

3. VALIDATION OF THE PROPOSED METHOD

The developed method for Axitinib and Avelumab was validated according to ICH guidelines of the parameters like Specificity, linearity, precision, accuracy, robustness, ruggedness, and forced degradation.

3.1.System suitability

System suitability was studied under each validation parameter by injecting six replicates of the standard solution. The system suitability parameters were shown in **Table no-1.02.**

Table-1.02 Results of System suitability data

System suitability parameter	Acceptance criteria	Drug name	
		Axitinib	Avelumab
% RSD	NMT 2.0	1.24	0.54
USP Tailing	NMT 2.0	1.02	1.06
USP plate count	NLT 2000	3471	7885

3.2. Specificity

i) Placebo interference:

Specificity was the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these include impurities, degrades, matrix, etc. It was observed that there is no interference at a retention time of Avelumab and Axitinib peaks with placebo peak and the results are summarized in **Table no-1.03 and Figure no-1.01-1.02.**

Table-1.03 Results of Specificity Study

Name of the solution	Retention time
Blank	No peak
Placebo	No peak
Axitinib	3.122 min
Avelumab	4.401 min

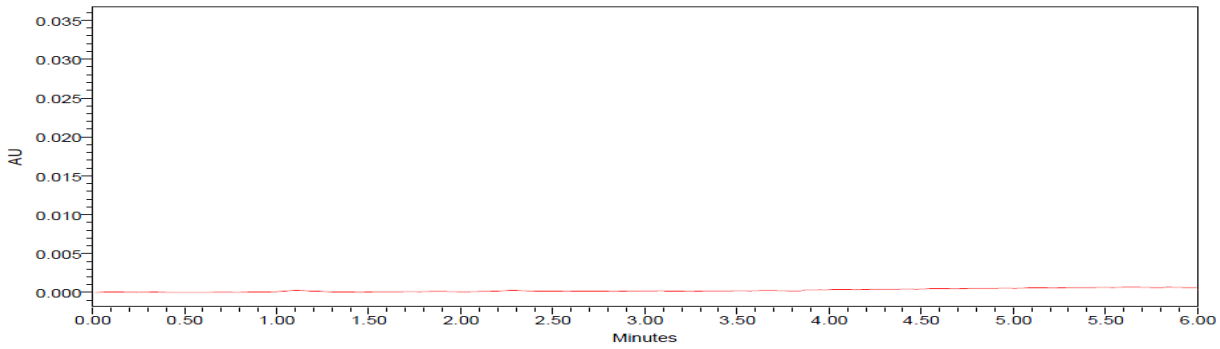

Fig.1.01 Chromatogram of blank

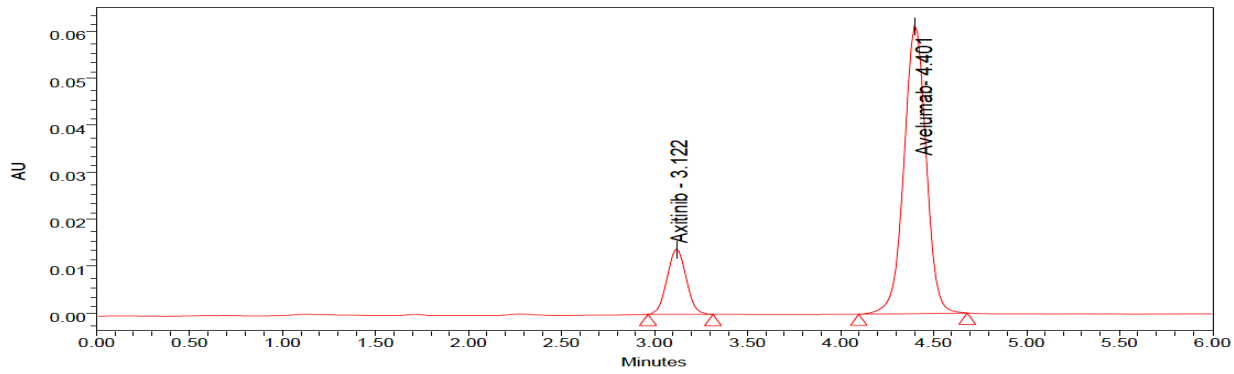

Fig.1.02 Chromatogram of Standard

ii) Forced degradation studies

To determine the analytical method and assay for the study of stability indicating method in the formulation of axitinib and Avelumab studied under various stress conditions to conduct forced degradation studies. Forced degradation conditions such as acidic, basic, peroxide, hydrolysis, reduction, and thermal stress were studied at 1N concentration levels. The discovery of such conditions is shown in **Tableno-1.04 & Figure no-1.03-1.10.**

Table-1.04 Results of forced degradation

Degradation results	**Axitinib**		**Avelumab**	
	Area	**% Degradation**	**Area**	**% Degradation**
Control	2154798	0.01	3245896	0.02
Acid	1859234	13.72	2746875	15.37
Base	1875124	12.98	2754102	15.15
Reduction	1820156	15.53	2813257	13.33
Hydrolysis	1846589	14.3	2789631	14.06
Peroxide	1864577	13.47	2865247	11.73
Thermal	1902342	11.72	2894012	10.84

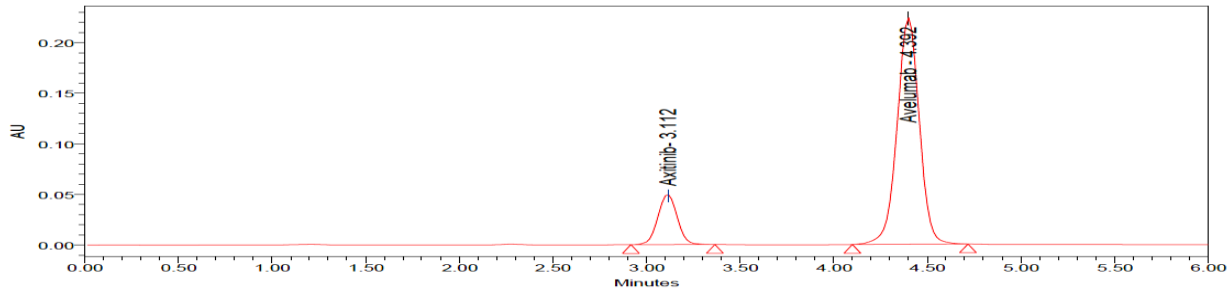

Fig.1.03 Chromatogram of control

a) Acid degradation:

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 mL of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup to the mark with diluents. Using a 0.45 syringe filter, filter the solution.1 ml of sample is moved to a 10 ml volumetric flask, along with 1 ml of 1N HCl, and the mixture is left to sit for 15 minutes. After 15 minutes, apply 1ml of 1N NaOH and dilute to the desired strength with diluents.

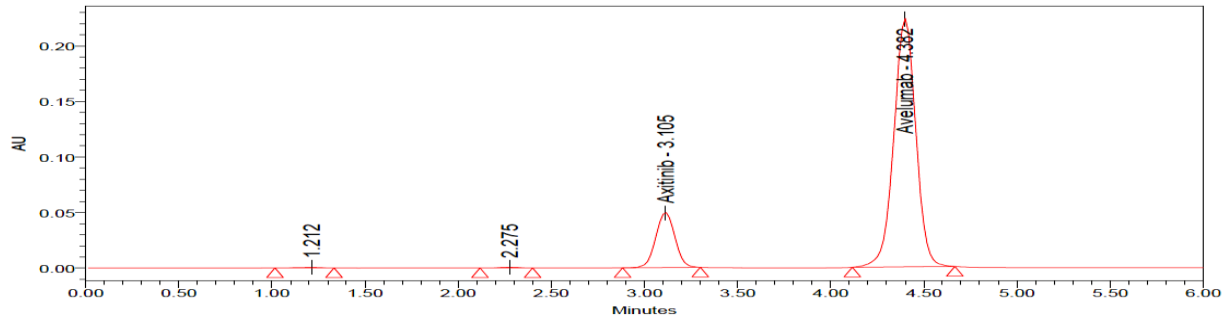

Fig.1.04 Chromatogram of acid degradation

b) Alkaline Degradation:

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 mL of diluents and sonicate for 30 minutes to fully dissolve the contents and makeup to the mark with diluents. Using a 0.45 syringe filter, filter the solution.1 ml of sample is moved to a 10 ml volumetric flask, along with 1 ml of 1N NaOH, and the mixture is left to sit for 15 minutes. After 15 minutes take 1ml of 1N HCl and dilute to the desired strength with diluents.

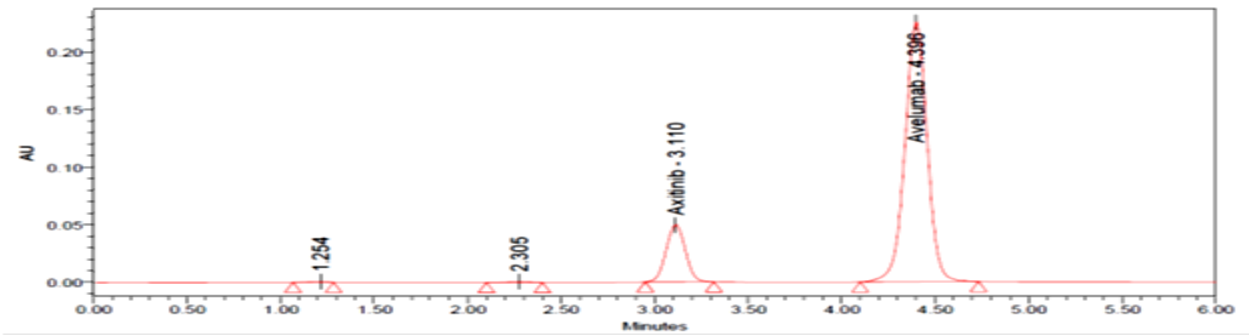

Fig.1.05 Chromatogram of alkali degradation

c) Peroxide Degradation:

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 mL of diluents, sonicate for 30 minutes, and makeup to the mark with diluents. Using a 0.45 µsyringe filter, filter the solution.1 ml of sample is taken into a 10 ml volumetric flask and add 0.3 ml of 30% hydrogen peroxide and make upto the volume with diluents.

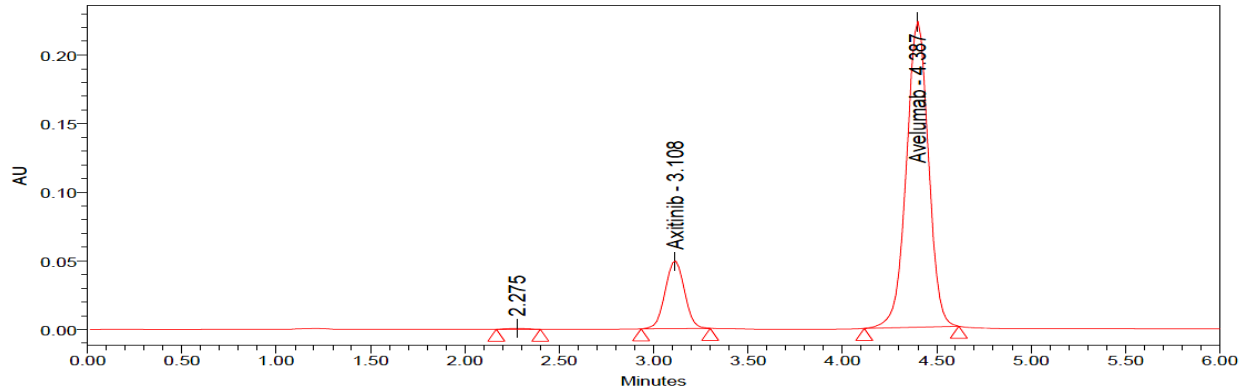

Fig.1.06 Chromatogram of peroxide degradation

d) Reduction Degradation:

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 mL of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup up the mark with diluents. Using a 0.45µ syringe filter, filter the solution.1 ml of sample is moved to a 10 ml volumetric flask, along with 1 ml of 30% sodium bi-sulfate solution, and diluted to the desired strength with diluents.

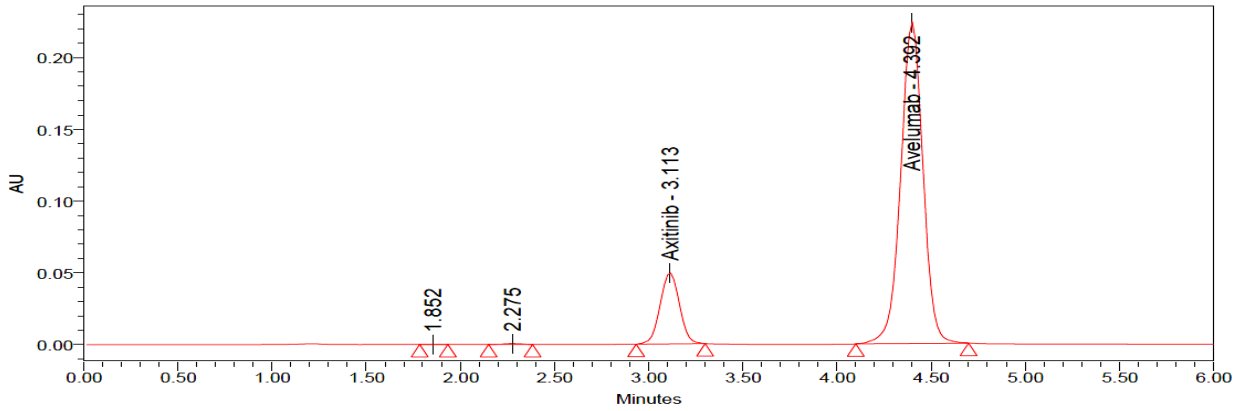

Fig.1.07 Chromatogram of Reduction degradation

e) Thermal Degradation

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 mL of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup up the mark with diluents. Using a 0.45µ syringe filter, filter the solution. The sample solution was placed in an oven at 105°C for 6 Hrs. The resultant solution was injected into the HPLC system.

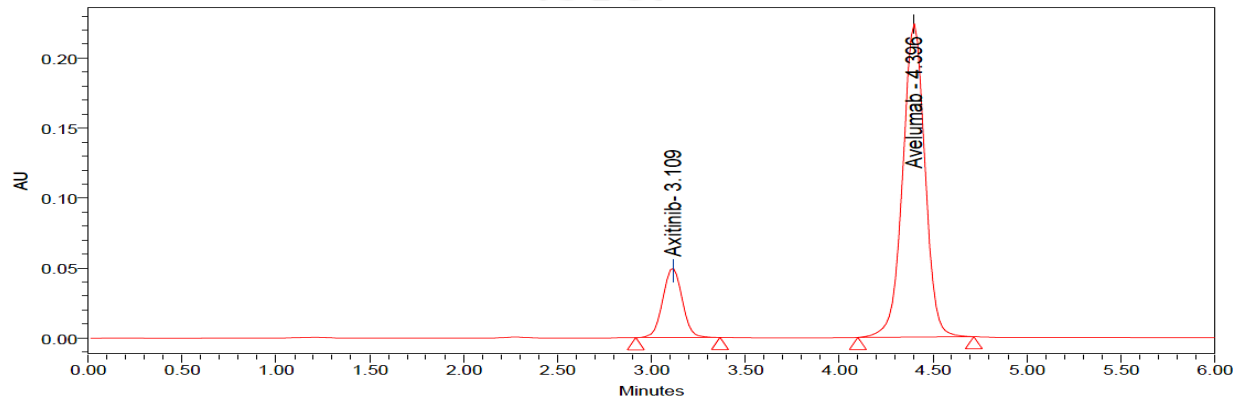

Fig.1.09 Chromatogram of thermal degradation

f) Hydrolysis Degradation:

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 ml of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup up the mark with diluents. Using a 0.45µ syringe filter, filter the solution 1ml of sample transferred into 10 ml volumetric flask, add 1 ml of water, and made up to the mark with diluents.

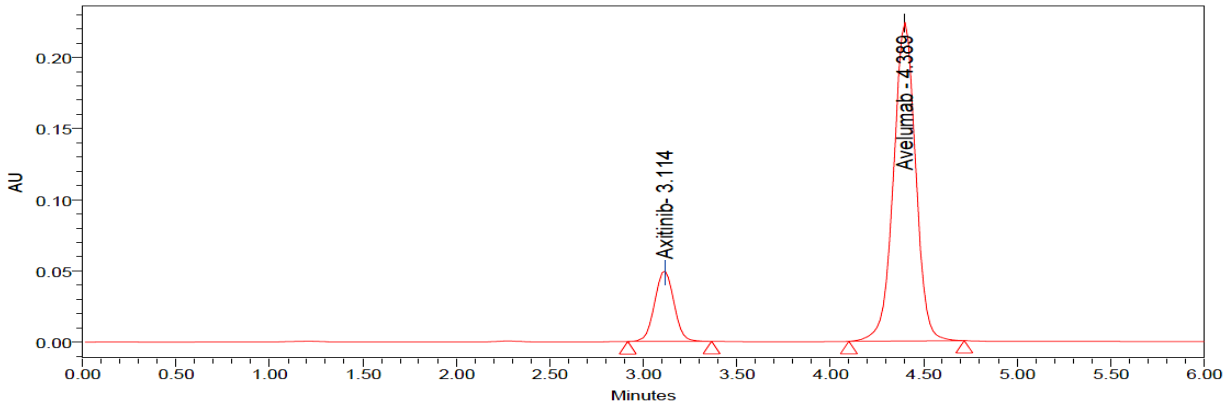

Fig.1.10 Chromatogram of hydrolysis degradation

3.3 Linearity

Preparation of linearity stock solution:

Working standards of 5 mg Axitinib and 20 mg Avelumab must be correctly weighed. These standards were put into a 100 ml volumetric flask, add 70mL of diluents, and sonicated for 10 minutes to dissolve the contents before being made up to the mark with the same diluents. The Linearity results are shown in **Table no-1.05 and Figure no-1.11-1.19.**

Preparation of linearity-10% solution: Take 0.5 ml of the above stock solution into a 50 ml volumetric flask and made it up to the mark with diluents.

Preparation of linearity-25% solution: Take 1.25 ml of the stock solution into the 50 ml volumetric flask and made it up to the mark with diluents.

Preparation of linearity-50% solution: Take 2.5 ml of the stock solution into the 50 ml volumetric flask and made it up to the mark with diluents.

Preparation of linearity-75% solution: Take 3.75 ml of the stock solution into the 50 ml volumetric flask and made it up to the mark with diluents.

Preparation of linearity-100% solution: Take 5 ml of the stock solution into a 50 ml volumetric flask and made it up to the mark with diluents.

Preparation of linearity-125% solution: Take 6.25 ml of the stock solution into a 50 ml volumetric flask and made it up to the mark with diluents.

Preparation of linearity-150% solution: Take 7.5 ml of the stock solution into a 50 ml volumetric flask and made it up to the mark with diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak areas concentration and calculate the correlation coefficient value. The response of the drug was found to be linear in the concentration range of 2-30ug/ml and the correlation coefficient was 0.999.

Acceptance criteria: The correlation coefficient should not be less than 0.999.

Table-1.05 Results of linearity

Linearity	**Axitinib**		**Avelumab**	
	Conc. (µg/ml)	**Area of analyte**	**Conc. (µg/ml)**	**Area of analyte**
Linearity-1	0.50	365098	2.00	336524
Linearity-2	1.25	624785	5.00	915181
Linearity-3	2.50	1200156	10.00	1748692
Linearity-4	3.75	1726425	15.00	2544693
Linearity-5	5.00	2174715	20.00	3265524
Linearity-6	6.25	2784593	25.00	4030598
Linearity-7	7.50	3375144	30.00	5036529
Slope	443300.01		163895.36	
Intercept	30330.08		42617.13	
CC	0.99929		0.99927	

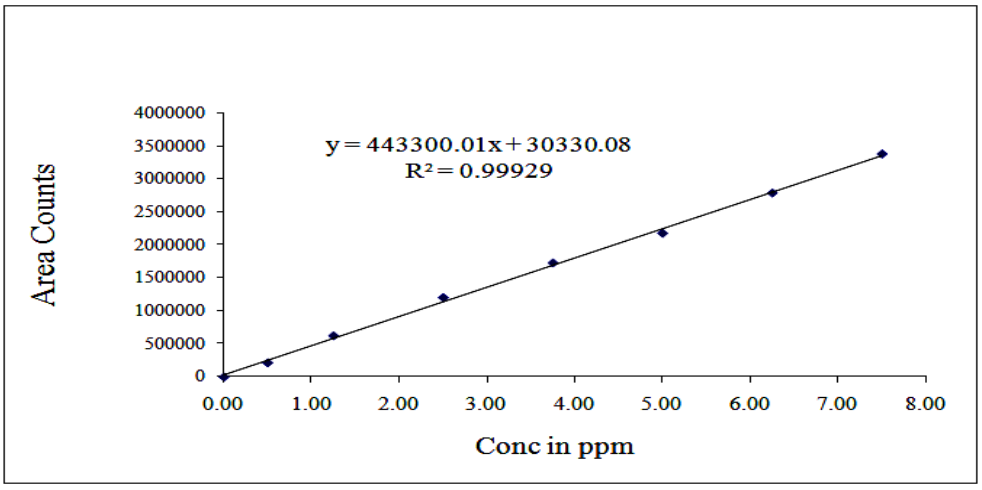

Fig.1.11 Linearity plot of Axitinib

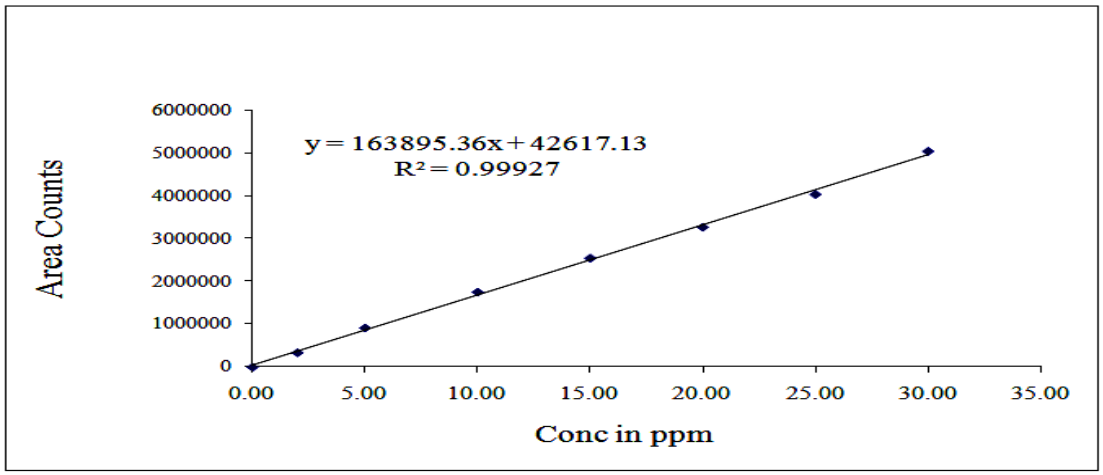

Fig.1.12 Linearity plot of Avelumab

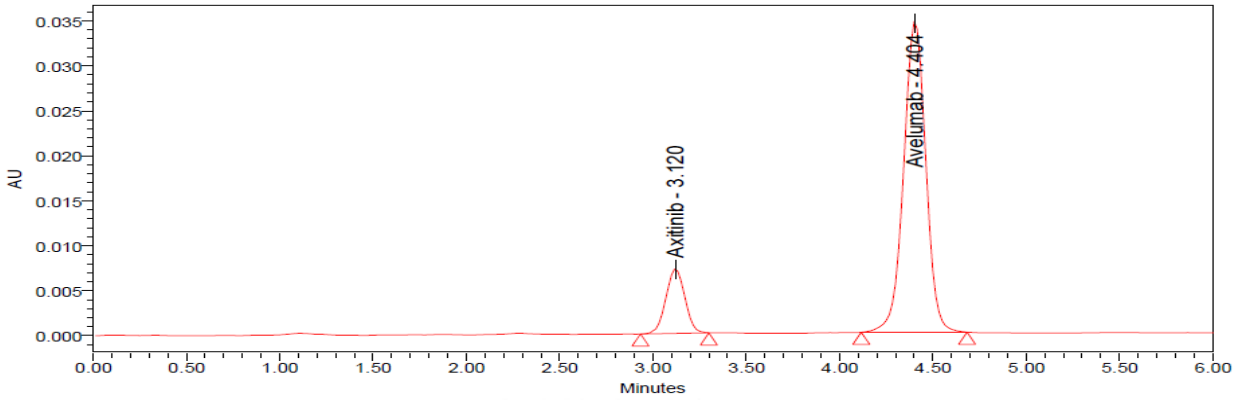

Fig.1.13 Chromatogram of linearity-10%

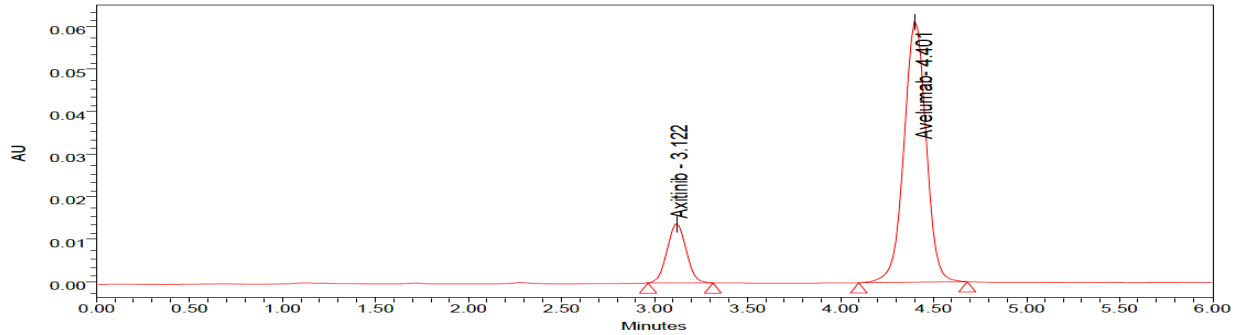

Fig.1.14 Chromatogram of linearity-25%

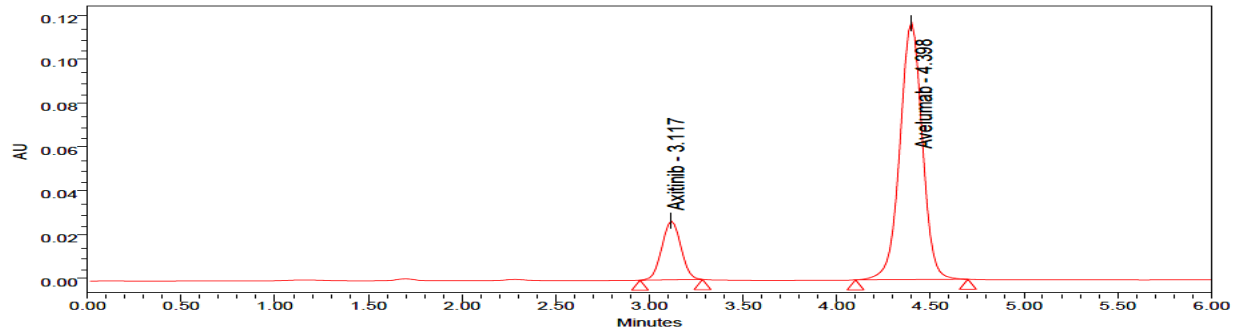

Fig.1.15 Chromatogram of linearity-50%

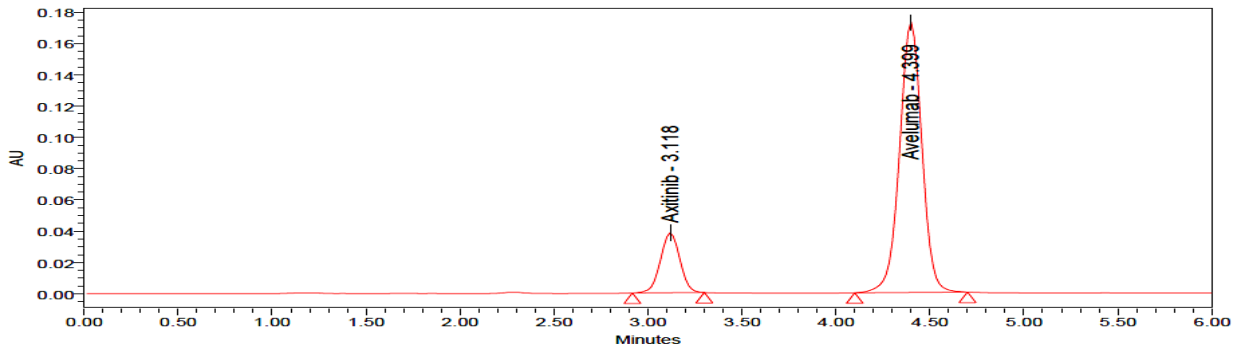

Fig.1.16 Chromatogram of linearity-75%

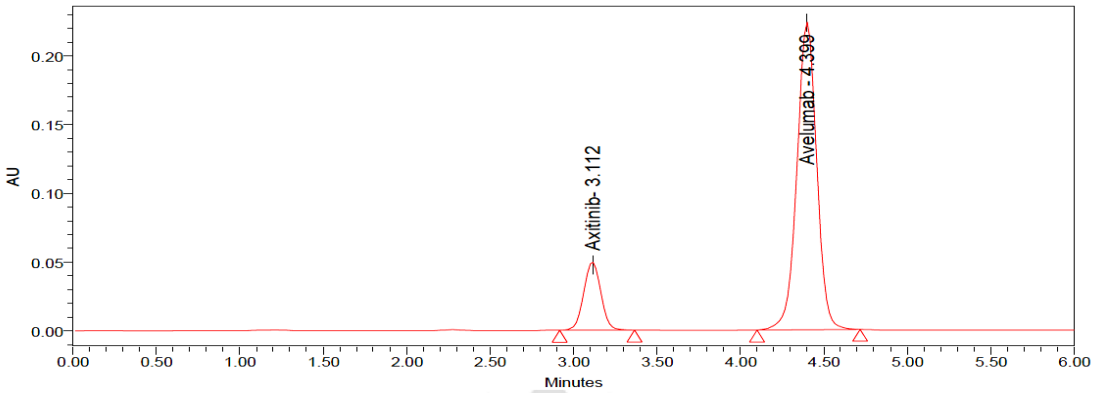

Fig.1.17 Chromatogram of linearity-100%

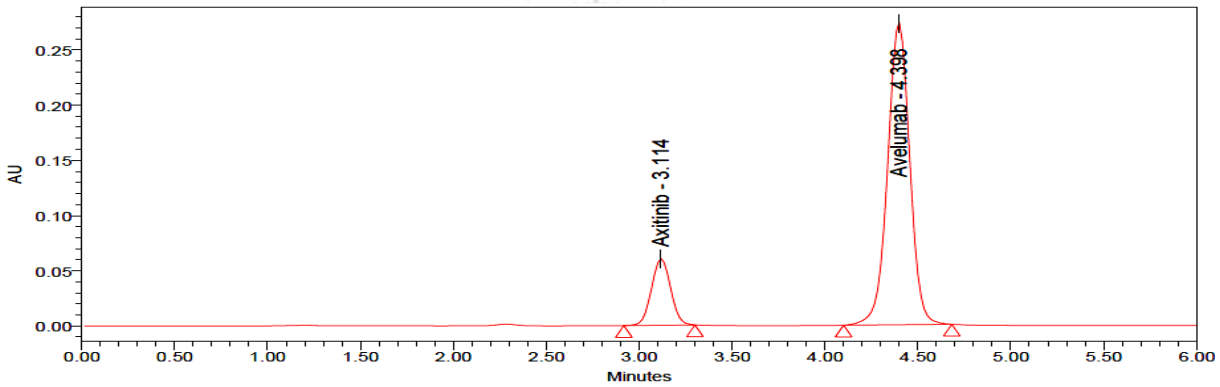

Fig.1.18 Chromatogram of linearity-125%

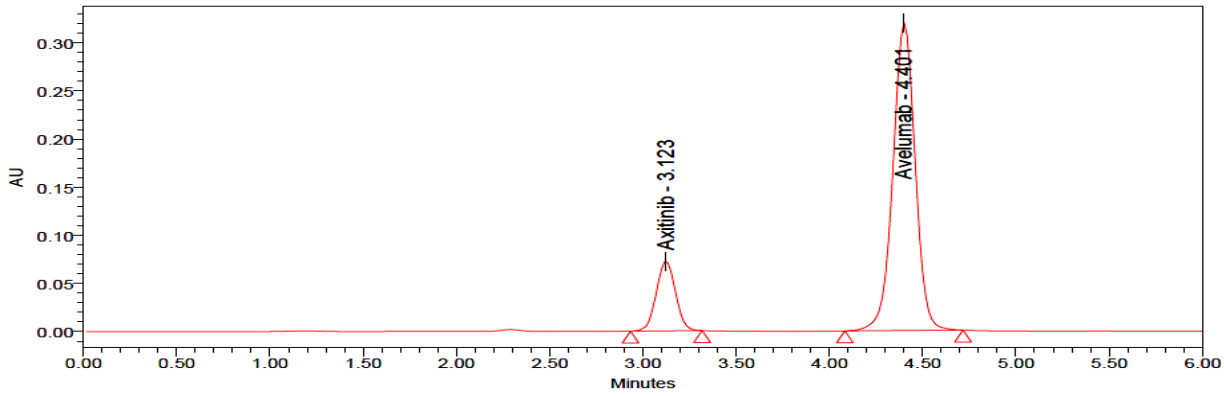

Fig.1.19 Chromatogram of linearity-150%

3.4 Accuracy

The accuracy of the method was determined by a known amount of standard drug added to a fixed amount of pre-analyzed capsule solution with spiking levels of 50%,100%, and 150%. Percentage recovery was calculated by comparing the area before and after the addition of the standard drug. The average %recovery and the %RSD should be within limits.

Preparation of 50% solution:

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample and add about 70 mL of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup to the mark with diluents. Further, take 2.5 ml of the above solution and transferred it into a 50 ml volumetric flask, and made it up to the mark with diluents.

Preparation of 100% solution:

In a 100 ml volumetric flask, measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 ml of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup up the mark with diluents. Further, take 5 ml of the above solution and transferred it into a 50 ml volumetric flask, and made it up to the mark with diluents.

Preparation of 150% solution:

In a 100 ml volumetric flask measure correctly 5 mg equivalent weight of Axitinib and 20 mg equivalent weight of Avelumab sample. Add about 70 mL of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup up the mark with diluents. Further, Take 7.5 ml of the above solution and transferred it into a 50 ml volumetric flask, and made it up to the mark with diluents.

Procedure:

Recovery of Axitinib and Avelumab was determined at three different concentration levels and inject each level into the chromatographic system. The mean recovery was 99.3-100.6%. The results of Accuracy are summarized in **Table no-1.06 and Figure no:1.20-1.23.**

Acceptance criteria: % recovery for each level should be 98-102%.

Table-1.06 Results of accuracy

Axitinib					Avelumab			
Spiking level (%)	**Amount of API added (ppm)**	**Amount of API found (ppm)**	**%Recovery**	**% Mean recovery**	**Amount of API added (ppm)**	**Amount of API found (ppm)**	**%Recovery**	**%Mean recovery**
50%	2.5	2.5	101.2	100	10	9.93	99.3	100
	2.5	2.5	99.6		10	9.97	99.7	
	2.5	2.5	99.2		10	10.14	101.4	
100%	5	5	99.4	99.9	20	19.96	99.3	99.8
	5	5	100.4		20	19.97	99.9	
	5	5	99.8		20	20.09	100.5	
150%	7.5	7.5	101.4	100.1	30	30.16	100.5	100.4
	7.5	7.5	99.7		30	30.02	100.1	
	7.5	7.5	100.1		30	30.07s	100.2	

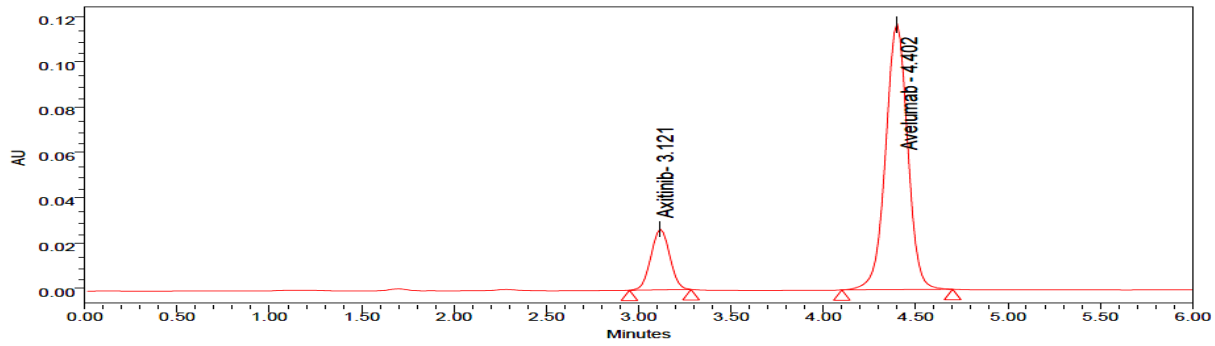

Fig.1.20 Chromatogram for accuracy at 50% spike level

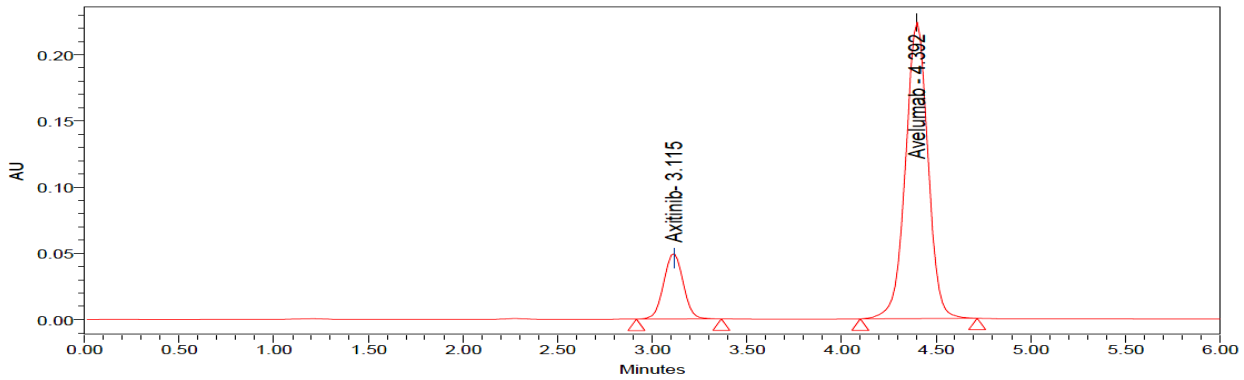

Fig.1.21 Chromatogram for accuracy at 100% spike level

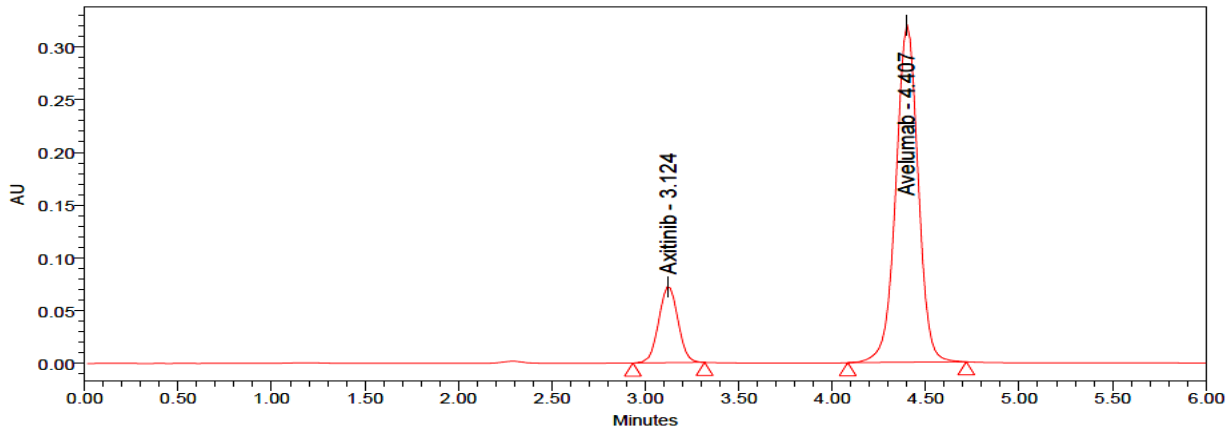

Fig.1.23 Chromatogram for accuracy at 150% spike level

3.5 Precision:

Precision is the degree of repeatability of an analytical method under operation conditions. Precision is of three types.

a) System precision

b) Method precision

c) Intermediate precision

a) System precision:

System precision is checked by using standard chemical substances to ensure that the analytical system is working properly. In this peak area and % drug of six determinations should be measured and % RSD should be measured.

b) Method precision:

The precision of an analytical technique is the degree of closeness of agreement between the series of measurements from samplings. The process of Method precision was performed by injecting six individual injections of Axitinib and Avelumab. In method precision, a homogeneous sample of a single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch.

In this analyze the sample six times and measure the % RSD. The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 20 ppm of Avelumab and Axitinib.

c) Intermediate precision:

Intermediate Precision of assay method was conducted on Axitinib and Avelumab using two different systems by different analysts using the different columns and analyzed under Day1 and Day2 similar conditions as per the test method.

System precision

To study the system precision, six replicates of the standard were injected into the HPLC system. The system suitability parameters are evaluated and found to be within the limits. The %RSD of Axitinib was 1.24% and Avelumab was 0.54%. The results of system precision of Axitinib and Avelumab are summarized in **Table no-1.07** and the chromatograms are shown in **Figure no-1.24-1.29.**

Table-1.07 Results of system precision

S. No	Axitinib		Avelumab	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	20	3265914	5	2185476
2	20	3274718	5	2154896
3	20	3225043	5	2130659
4	20	3265698	5	2175478
5	20	3256849	5	2125685
6	20	3264583	5	2184798
Average	3258801	2159499		
Std dev	17483.470	26707.648		
% RSD	0.54	0.537		

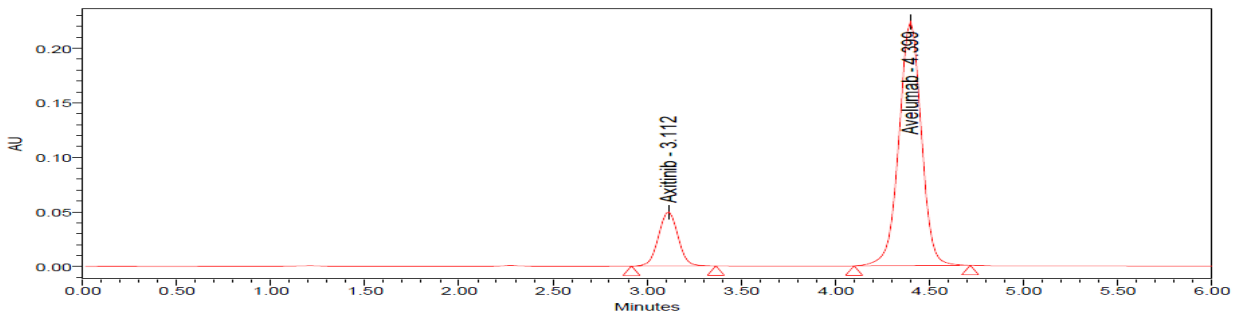

Fig.1.24 Chromatogram of standard-1

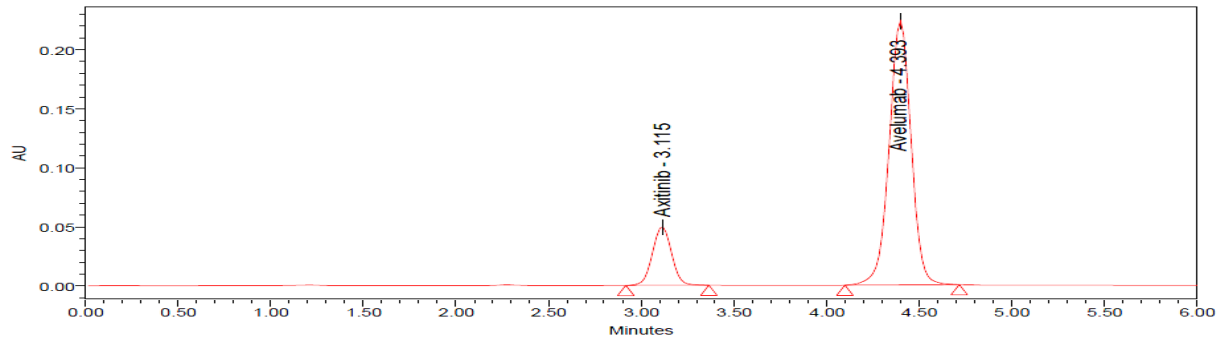

Fig.1.25 Chromatogram of standard-2

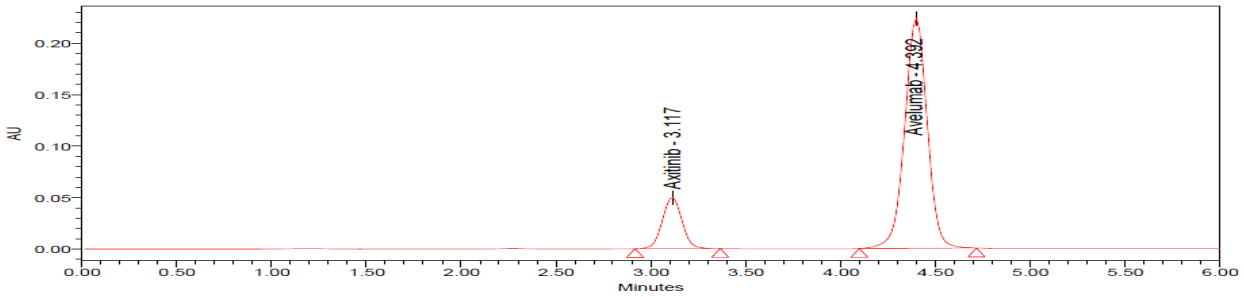

Fig.1.26 Chromatogram of standard-3

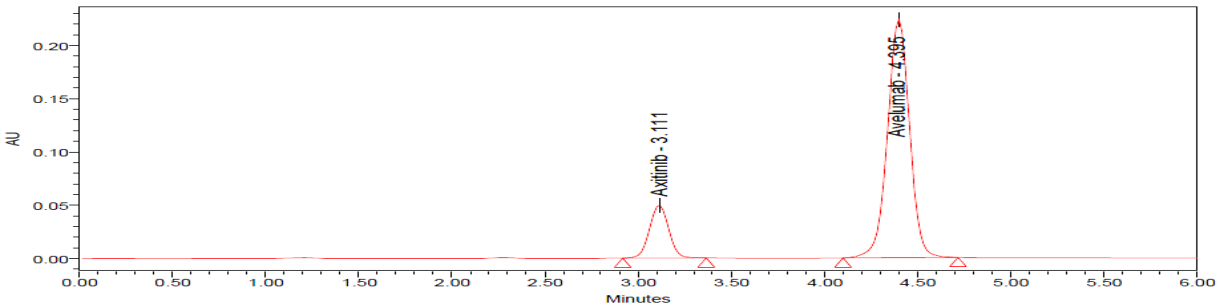

Fig.1.27 Chromatogram of standard-4

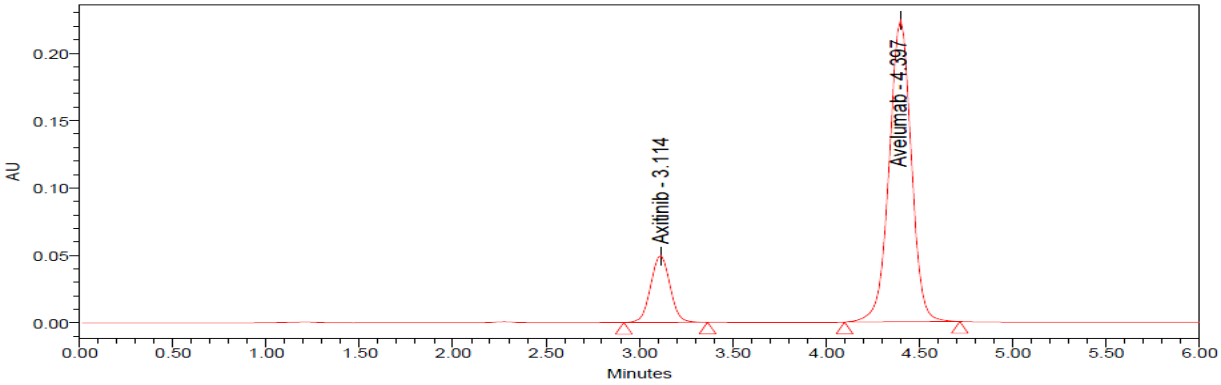

Fig.1.28 Chromatogram of standard-5

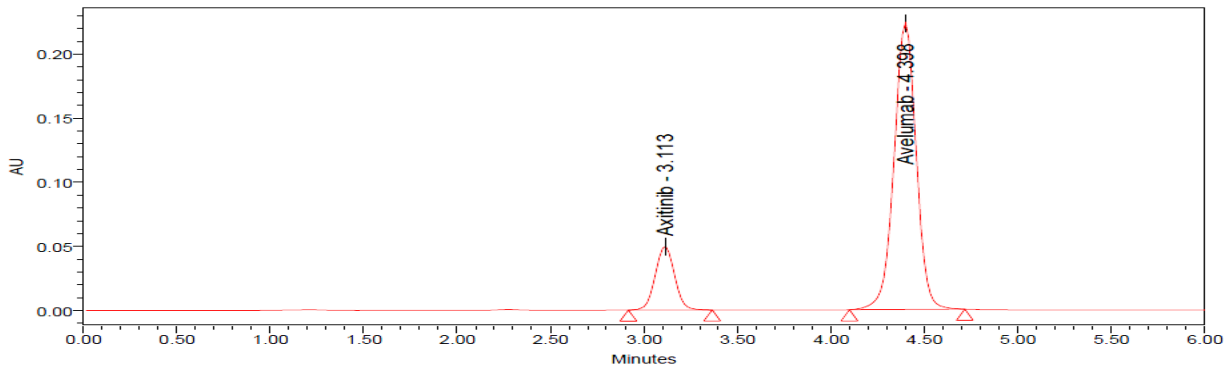

Fig.1.29 Chromatogram of standard-6

a) Method precision

Method precision was carried out by injecting six samples from the Axitinib and Avelumab and the %RSD was calculated. The %RSD of the six preparations in the Method precision was found to be 0.38. The results of Method precision were summarized in **Table no-1.08** and the chromatograms are shown in **Figure no-1.30.**

Table-1.08 Results of method precision

S. No.	Area for Axitinib	Area for Avelumab
1	2136259	3256478
2	2154987	3214562
3	2136502	3232588
4	2165471	3225964
5	2145985	3250124
6	2156374	3296983
Average	2149263	3246117
Std dev	11736.45	29310.92
%RSD	0.57	0.91

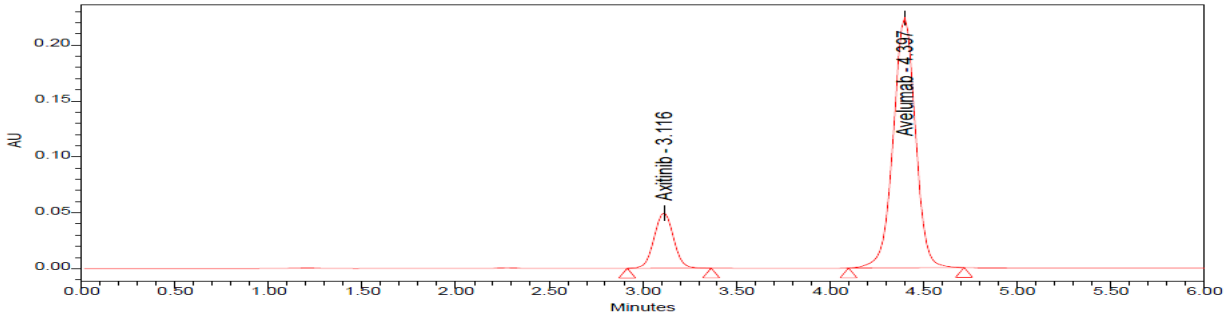

Fig.1.30 Chromatogram of method precision

b) Intermediate precision:

The intermediate precision of the assay method was carried out by using the same sample of Avelumab and Axitinibusing two different systems by using different analysts using the different columns and analyzing under Day 1 and Day 2 similar conditions as per the test method. The results for the intermediate precision were summarized in **Table no-1.09** and the chromatograms are shown in **Figure no-1.31**.

Table-1.09 Results of Intermediate Precision

S. No.	Axitinib		Avelumab	
	Day-1	Day-2	Day-1	Day-2
1	2167896	2154798	3261915	3136523
2	2189863	2132569	3205987	3265478
3	2129674	2142151	3215647	3274965
4	2184897	2142516	3231496	3254102
5	2145876	2134798	3267487	3235980
6	2155862	2100369	3256201	3205402
Average	2163945		3228742	
Std dev	25759.08		51440.68	
%RSD	1.19		1.59	

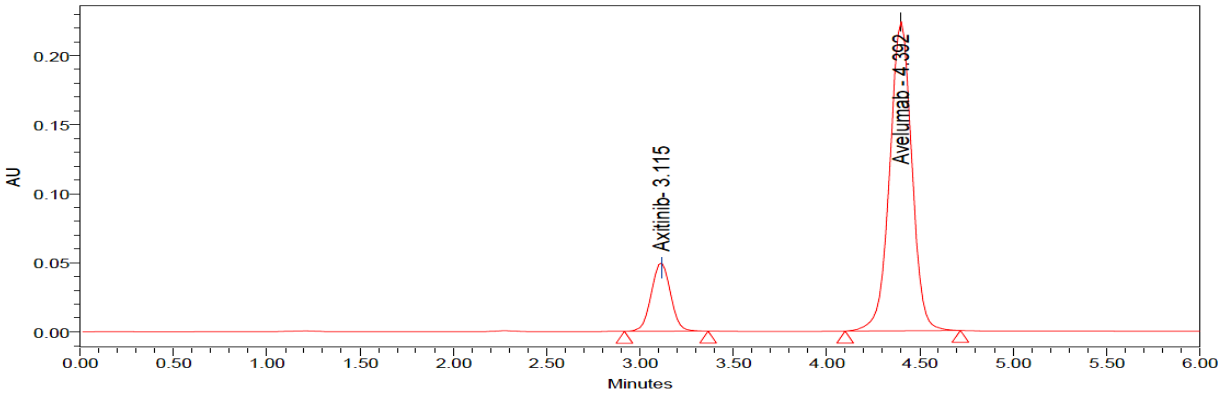

Fig.1.31Chromatogram of intermediate precision

3.6 LOD and LOQ

The limit of detection and limit of quantification of the drug was calculated by using the following equation as per ICH guidelines. The Limit of detection and Limit of quantification

were evaluated by serial dilution of Axitinib andAvelumab stock solution to determine a signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ. The concentration of LOD and LOQ for Axitinib and Avelumab were listed in **Table no-1.10** and the chromatograms are shown in **figure no-1.32-1.33**.

$$LOD = 3.3\ \sigma/s \quad LOQ = 10\ \sigma/s$$

Table-1.10 Sensitivity parameter values

Name of the drug	LOD (µg/ml)	S/N	LOQ (µg/ml)	S/N
Avelumab	0.025	7	0.825	26
Axitinib	0.0063	5	0.0206	24

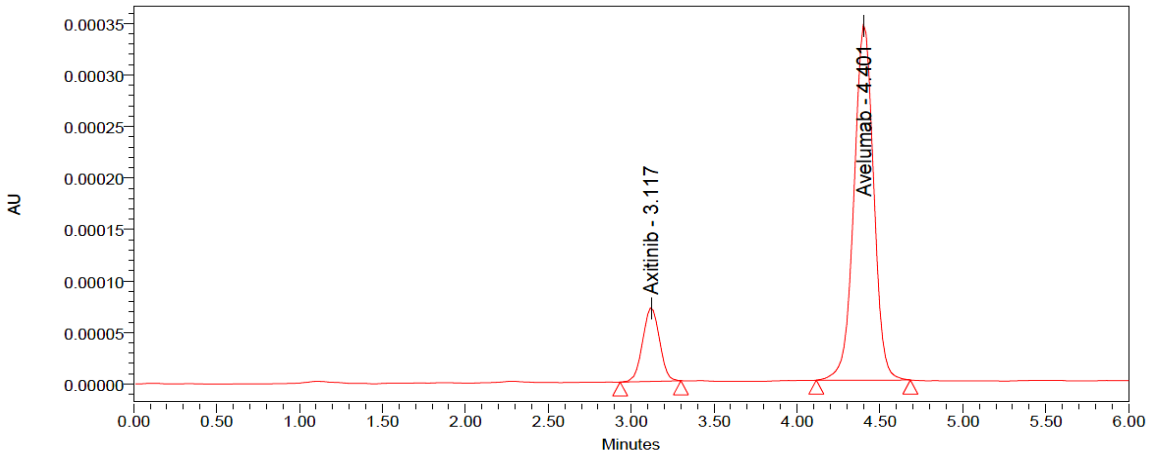

Fig.1.32 Chromatogram of LOD

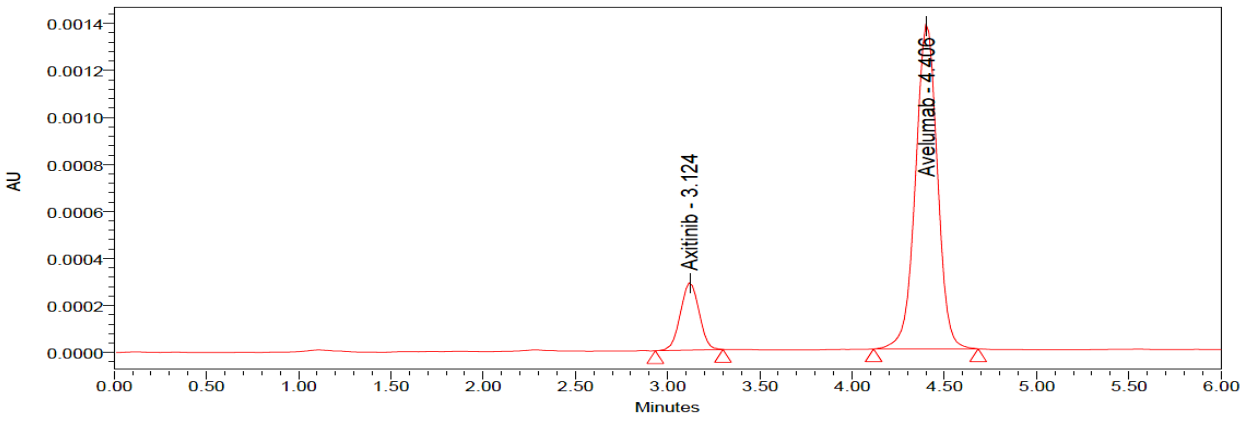

Fig.1.33Chromatogram of LOQ

3.7 Robustness

As part of the robustness, deliberate changes in the flow rate, mobile phase, and organic phase composition were made to evaluate the impact on the method. The result of the Robustness study of the developed assay method was established in **Table no-1.11and Figure no-1.34-1.37.**

A. The flow rate was varied from 0.8 ml/min to 1.2 ml/min

Standard solutions of 5ppm of Axitinib and 20ppm of Avelumab were prepared and analyzed under variable flow rate and it can be concluded that the variation in the flow rate affected the method significantly. Hence it indicates that the method is robust even with the change in the flow rate is ±2%.

B. Variation of organic phase ratio

A standard solution of 5 ppm of Axitinib and 20 ppm of Avelumab was prepared and analyzed under a variable mobile phase ratio. On the evaluation of the above results, it can be concluded that the variation in the mobile phase ratio affected the method significantly. Hence it indicates that the method is robust even by a change in the mobile phase is ±10%.

Table-1.11 Results of Robustness

Parameter name	% RSD	
	Axitinib	**Avelumab**
Flow rate(0.8 ml/min)	1.03	0.81
Flow rate(1ml/min)	1.02	s1.23
Flow rate (1.2 ml/min)	1.11	1.26
Organic solvent (-10%)	0.58	0.33
Organic Solvent	0.78	0.65
Organic solvent (+10%)	0.79	0.67

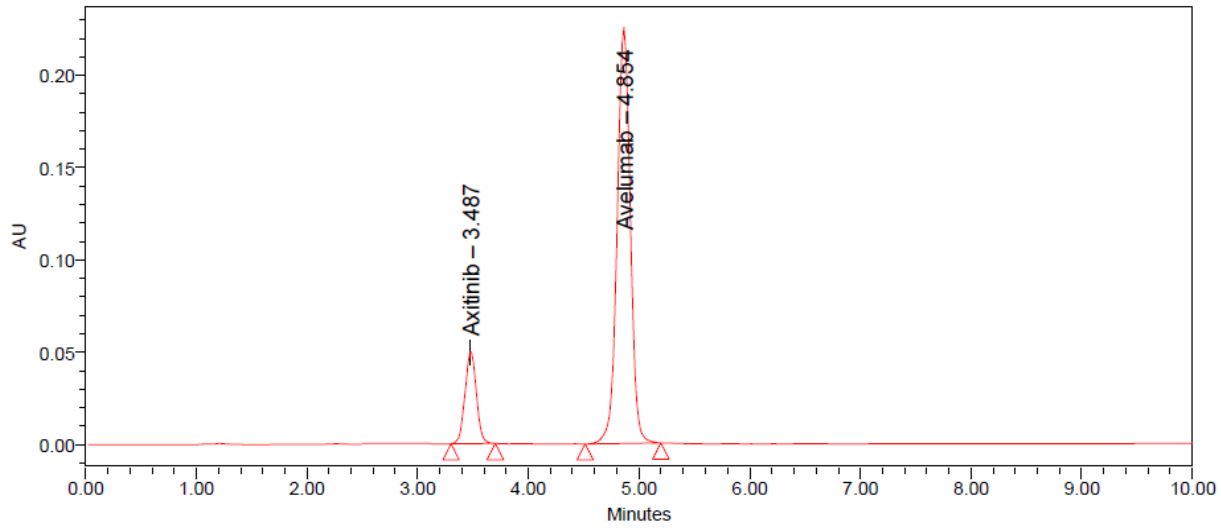

Fig.1.34 Chromatogram of flow minus

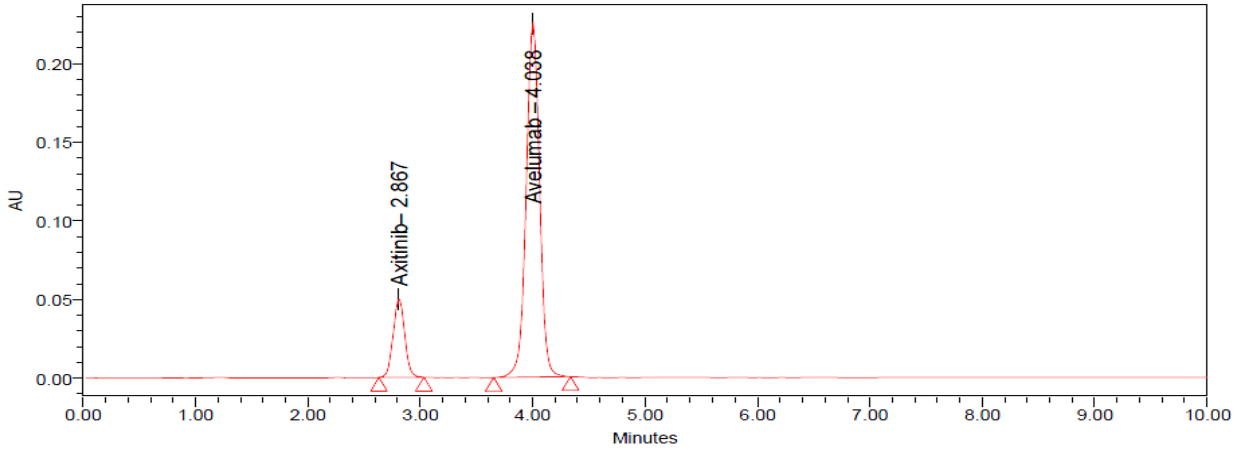

Fig.1.35Chromatogram of flow plus

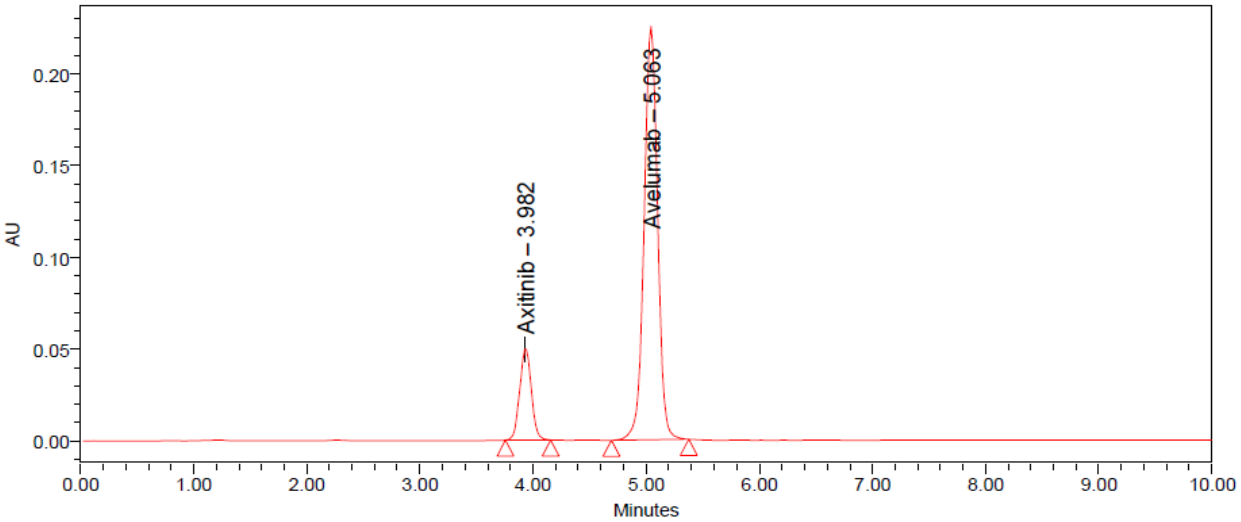

Fig.1.36 Chromatogram of organic minus

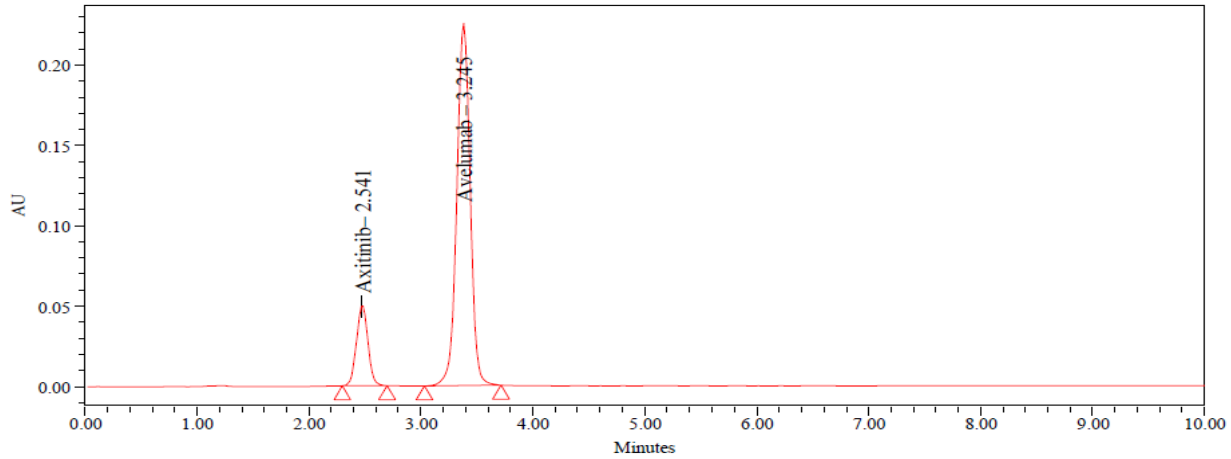

Fig.1.37Chromatogram of organic plus

3.8 CONCLUSION:

This method describes the quantification of Axitinib and Avelumab in bulk and pharmaceutical formulation as per ICH guidelines. The developed method was found to be accurate, precise, linear, and reliable. The advantage of the developed method was sample preparation is easy and low-cost reagents were used. The proposed RP-HPLC conditions ensure sufficient resolution and therefore the precise quantification of the compounds.

The Author developed a new stability indicating RP-HPLC method developed using symmetry C18 Column. A column with mobile phase Acetonitrile and Ortho phosphoric acid (OPA)Buffer (pH 4.0) in the ratio of (60:40) and run in isocratic mode. Flow rate was 1.0ml/min, with injection volume 10ul detection done by using a PDA detector at 222nm. The runtime was 10min and the Retention time of Axitinib and Avelumab are 3.158&4.429min which enables rapid quantification of many samples in routine and quality control analysis of Injection formulation.

The method was validated as per ICH guidelines by using various validation parameters like Specificity, linearity, accuracy, precision, robustness, and solution stability.

From the specificity study, it was concluded that the developed method was specific and the degradation studies under various conditions concluded that the degradation was observed in sufficient quantity and it was the stability-indicating method. Statistical analysis of the experimental results indicates that the precision and reproducibility of data are satisfactory. The developed chromatographic method was often effectively applied for routine analysis in drug research.

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