ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CAPMATINIB IN BULK AND TABLET DOSAGE FORM BY RP-HPLC METHOD

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Abstract

An HPLC method was developed and validated for the estimation of Capmatinib in bulk and pharmaceutical dosage form. The chromatographic system was equipped Agilent column 150 mm x 4.6 mm internal diameter with 5 micron particle size column and UV detector set at 218nm, in conjunction with a mobile phase of Methanol and phosphate buffer PH 5 in the ratio of (65:35) at a flow rate of 0.5 ml/min. The retention time of capmatinib was found to be 4.3 minute. The separation was performed at ambient temperature. The injection volume was 10µl. Linearity in concentration range of 10-50µg mL with regression 0.999. The Percentage recoveries were found in the range of 101.4– 101.8%. The proposed method was validated in accordance with ICH parameters the method is precise, accurate, selective and rapid.

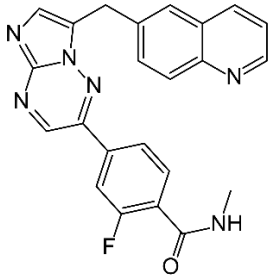
Key Words: *RP-HPLC, Capmatinib, Validation, and ICH.*

Introduction:

Capmatinib, also known as Tabrecta, is a drug used to treat adults with metastatic Non-Small Cell Lung Cancer (NSCLC) whose tumours contain an exon 14 skipping mutation in the MET gene, which codes for the membrane receptor HGFR, as confirmed by an FDA-approved test. Capmatinib and the Foundation One CDx assay as a companion diagnostic for capmatinib were authorised for medical use in the United States in May 2020.

NSCLC (Non-Small Cell Lung Cancer) is a disease in which malignant cancer cells originate in the lung tissues. Non-small cell lung cancer is the most frequent kind of lung cancer, accounting for up to 90% of all lung carcinomas. When healthy cells become aberrant and expand fast, NSCLC develops. One of the dangers of this type of cancer is that the cancer cells are likely to travel from the lungs to other organs and body parts. Cancer metastasis is comprised of a variety of events, with MET exon 14 skipping being recognised as a crucial step in carcinoma metastasis. MET exon 14 skipping mutations are detected in 3-4 percent of lung cancer patients.

Structure:



IUPAC Name:

2-fluoro-*N*-methyl-4-[7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2yl]benzamide.

Mechanism of Action:

Many malignancies, including non-small cell lung cancer, have been shown to have abnormal c-Met activity (NSCLC). Mutations that cause MET exon 14 to be skipped result in

the creation of a mutant c-Met protein that lacks a regulatory domain; these mutant proteins have a diminished ability to negatively regulate, resulting in a pathological increase in their downstream activity.

Capmatinib blocks c-Met-mediated phosphorylation of downstream signalling proteins, as well as cell proliferation, by inhibiting the phosphorylation of both wild-type and mutant versions of c-Met caused by the binding of its endogenous ligand, hepatocyte growth factor.

Materials and Methods:

Apparatus:

SIMADZU Prominence HPLC iLC2030 Plus

Materials and reagent:

All the chemicals used were of analytical and HPLC grade. An analytically pure sample for capmatinib was procured as a gift sample from the Nebulae Hi Tech Laboratory, Chennai –116

Solvents:

Methanol, phosphate buffer of HPLC grade and Milli Q water were used in analysis.

Preparation of Phosphate buffer

7.0 grams of Potassium dihydrogen Phosphate was weighed accurately and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. The pH was adjusted to 3.0 with Ortho phosphoric acid.

Preparation of mobile phase

Phosphate buffer 350 ml (35%) and 650 ml of Methanol HPLC (65%) was measured and mix well. Then the solution was degassing in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of Standard stock solution

About 10 mg Capmantinib was accurately weighed and transfer of into a 10 ml volumetric flask add about 7 ml of mobile phase and sonicate to dissolve it completely and make volume up to the mark with the mobile phase. The solution was observed to contain 1000 µg/ml.

SYSTEM SUITABILITY

Parameter	Capmantinib
Tailing factor	1.6
No. of Theoretical plates	2514.6
Retention time	4.349

Preparation of Sample solution

10 tablets of Capmantinib were weighed and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Capmantinib into a 10ml volumetric flask. Add about 7 ml of mobile phase and sonicate to dissolve it completely and make volume up to the mark with the mobile phase. Mix well and filter through 0.45 µm filter.

Linearity:

In this method, the aliquots of stock solution of Capmantinib (0.1-0.5 ml of 1000 µg/ml) were transferred into five 10 ml volumetric flask and make up to the mark with mobile phase. A solution contains 10, 20, 30, 40 and 50 µg/ml of Capmantinib in mobile phase. The solutions were injected and the chromatograms were recorded at 218 nm. It was found that the above concentration range was linear with the concentration range of 10-50 µg/ml. The peak area was plotted against concentration and the calibration curve was constructed.

Quantification of Formulation

20 µl of the standard, sample solution (30 µg/ml) was injected into the chromatographic system. Chromatogram was recorded and the peak area was measured. Then calculate the % purity by using the formulae.

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where,

AT = Peak Area of Capmantinib obtained with test preparation
AS = Peak Area of Capmantinib obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Recovery study:

Equivalent to 10 mg of Capmatinib tablet powder was accurately weighed and transfer into a three separate10 ml volumetric flask. Then 5mg, 10mg and 15mg (50%, 100%, 150%) of standard were accurately weighed and added. 7 ml of methanol was added and sonicate to dissolve it completely. Then it was made volume up to the mark with the same solvent. 0.3ml was pipette out from each flask and transferred to separate 10ml volumetric flask. Then the solution was made volume up to the mark with the same solvent. 20µl solution was injected in to the chromatographic system. Percentage recovery was calculated by using peak area.

Precision and Intermediate Precision

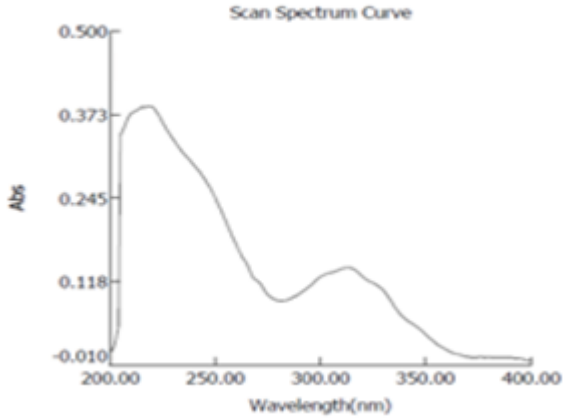
Pipette out 0.3 ml of the stock solution into a 10 ml volumetric flask and dilute up to the mark with mobile phase. Mix well and filter through 0.45 µm filter. The solution was observed to contain 30 µg/ml. The solution was injected for five times and the chromatogram was recorded. Peak area was used to calculate the %RSD value.

Robustness

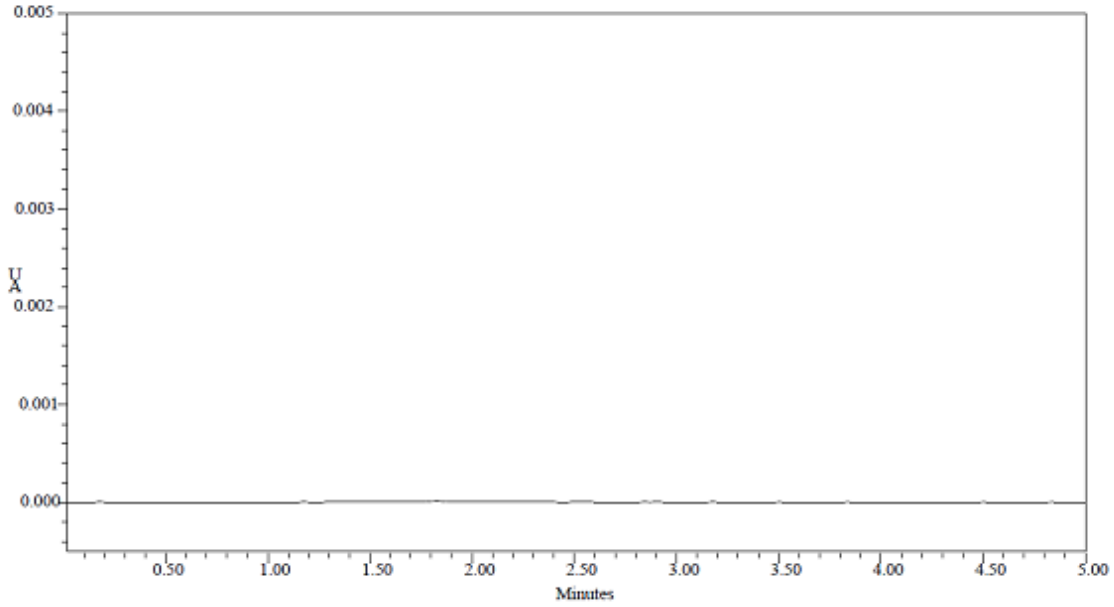
As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact of the method.

a. Flow rate was varied at 0.5-0.7 ml/min
b. Organic composition in the mobile phase was varied 60% - 50%.

Results &Discussion



UV Spectrum of Capmantinib in Methanol: Phosphate buffer pH 3.0 (55:45% v/v)

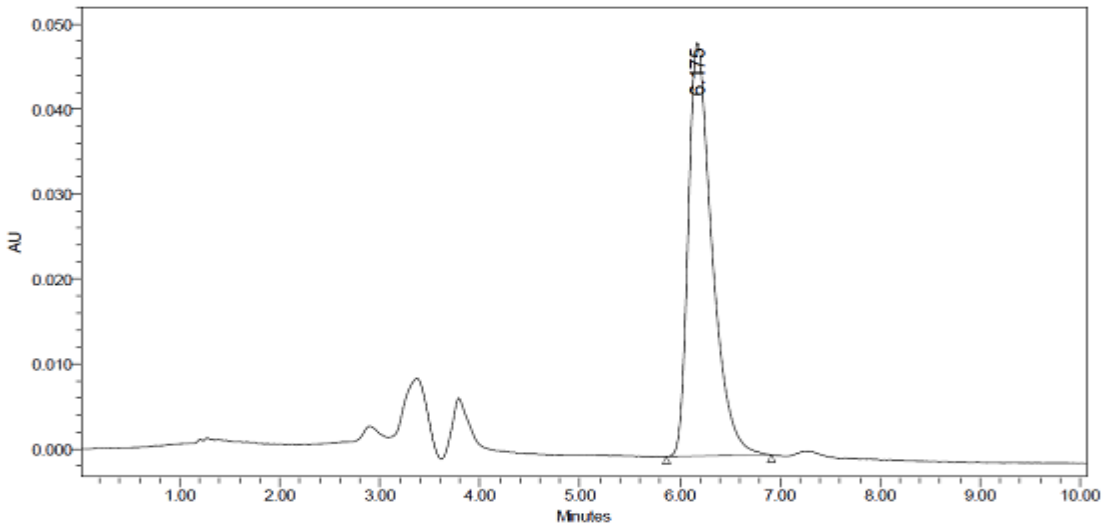


Blank

	Baseline Noise (mV)
1	0.052

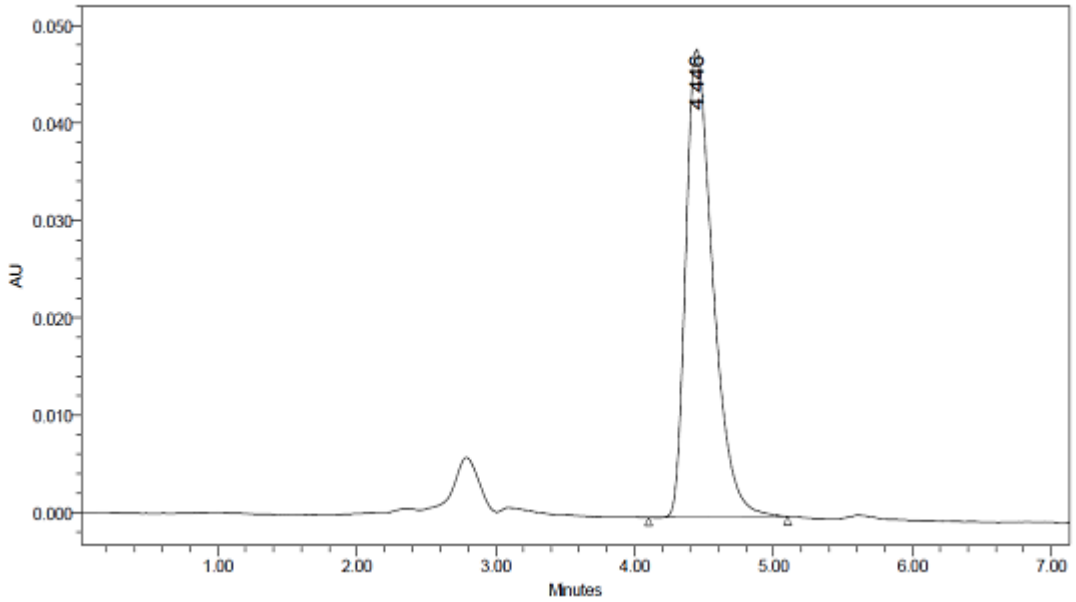
Initial separation conditions

Mode of operation	:	Isocratic
Stationary phase	:	C_{18} Column (150 mm x 4.6 mm i.d., 5µ)
Mobile phase	:	Methanol : Phosphate buffer pH 2.5
Ratio	:	50:50 % v/v
Detection wavelength	:	218 nm
Flow rate	:	0.5 ml/min
Temperature	:	Ambient
Sample volume	:	20µl



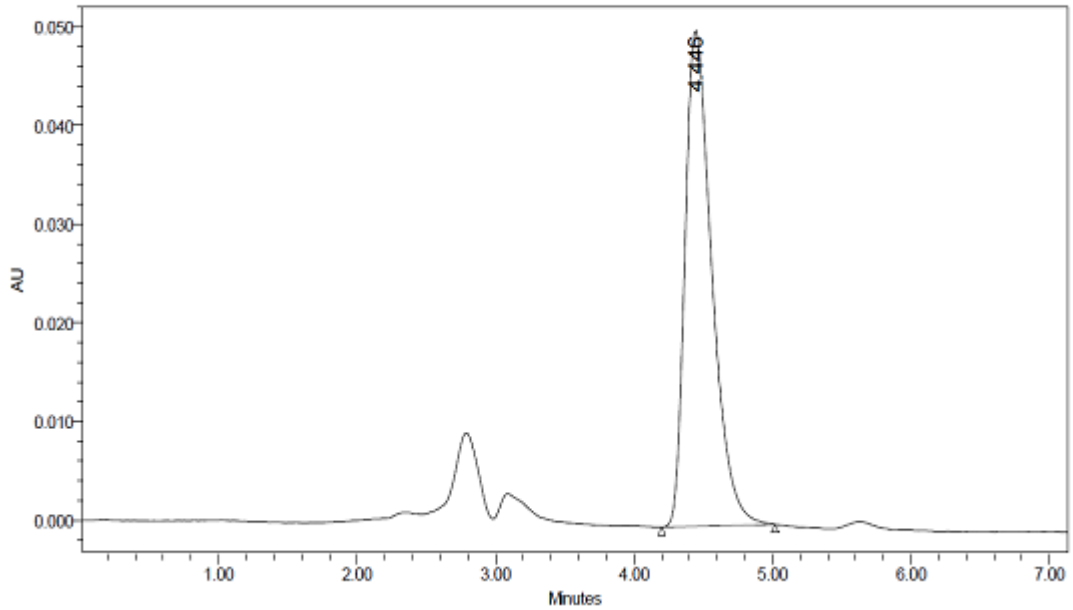
Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
6.175	804030	48852	3269.4	1.5

Trial-1 Methanol: Phosphate Buffer pH 2.5 (55:45% V/V)



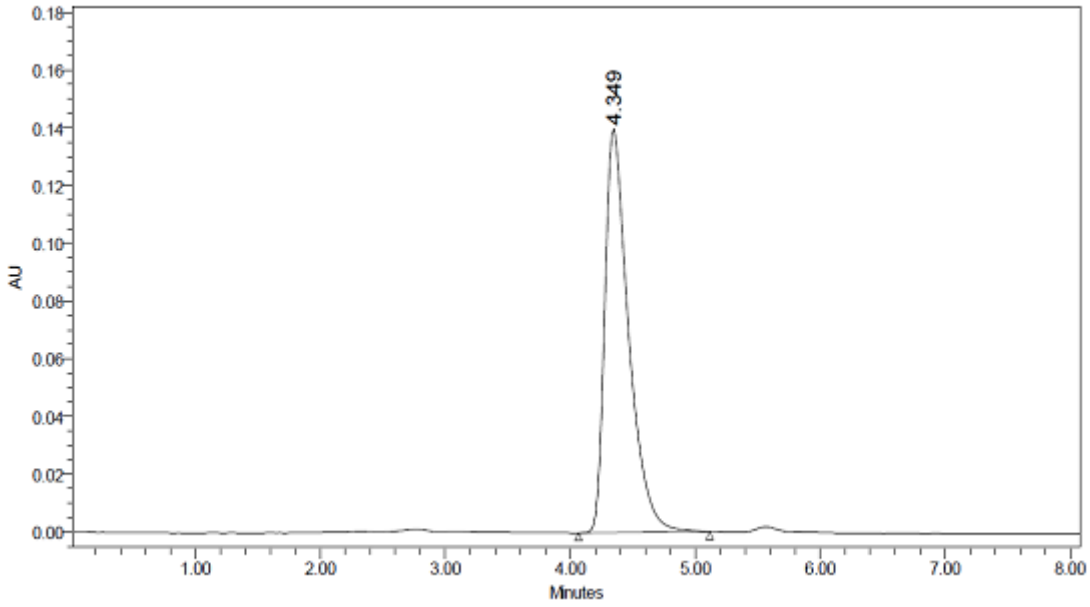
Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
4.446	650745	48094	2511.0	1.5

Trial-2 Methanol : Phosphate Buffer pH 3.0 (45:55% V/V)



Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
4.446	675129	50307	2527.2	1.5

Trial-3 Methanol: Phosphate Buffer pH 3.0 (50:50% V/V)



Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
4.349	1852501	139896	2514.6	1.6

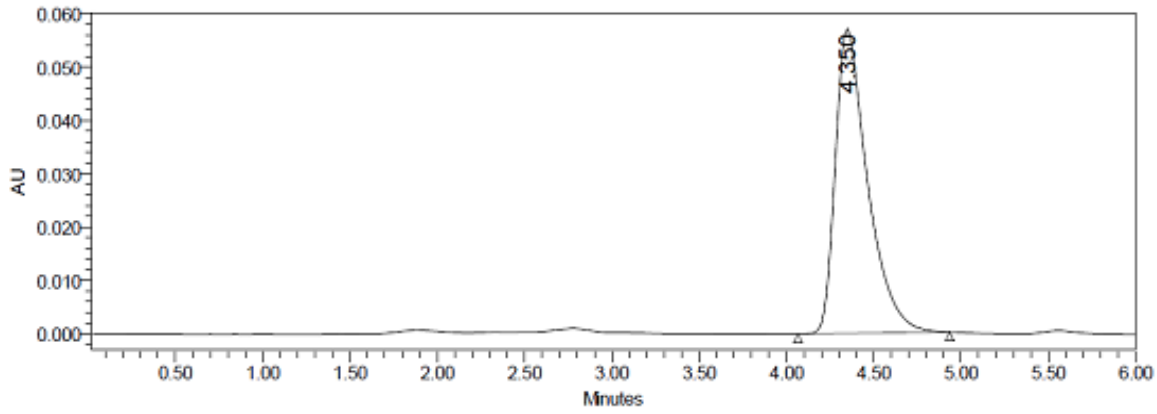
Methanol: Phosphate Buffer pH 3.0 (65:35% V/V)

Optimized Chromatogram

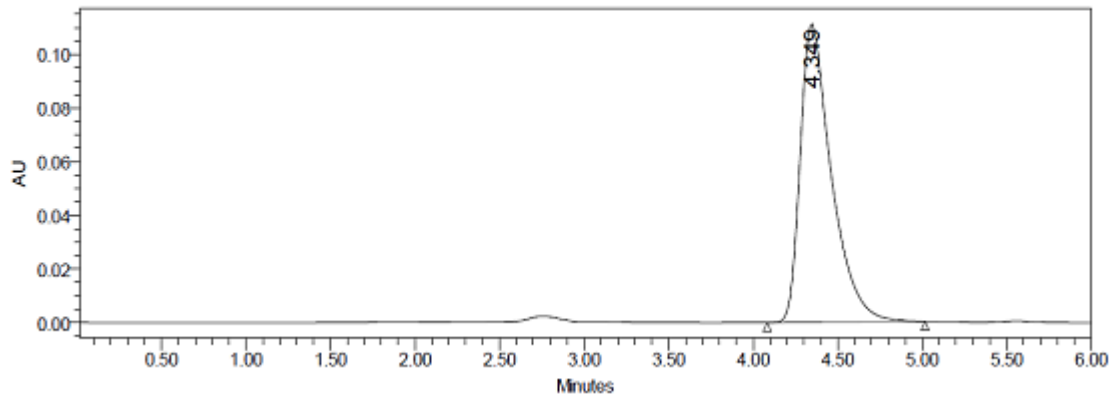
Optimized Chromatographic Conditions

Mode of operation	:	Isocratic
Stationary phase	:	C_{18} Column (150 mm x 4.6 mm i.d., 5µ)
Mobile phase	:	Methanol : Phosphate buffer pH 3.0
Ratio	:	65:35 % v/v
Detection wavelength	:	218 nm
Flow rate	:	0.6 ml/min
Temperature	:	Ambient
Sample volume	:	20µl

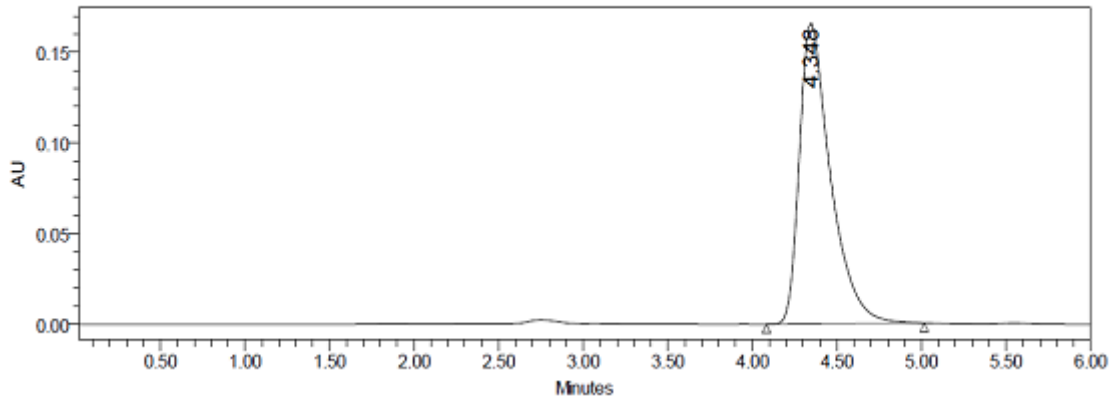
Linearity:



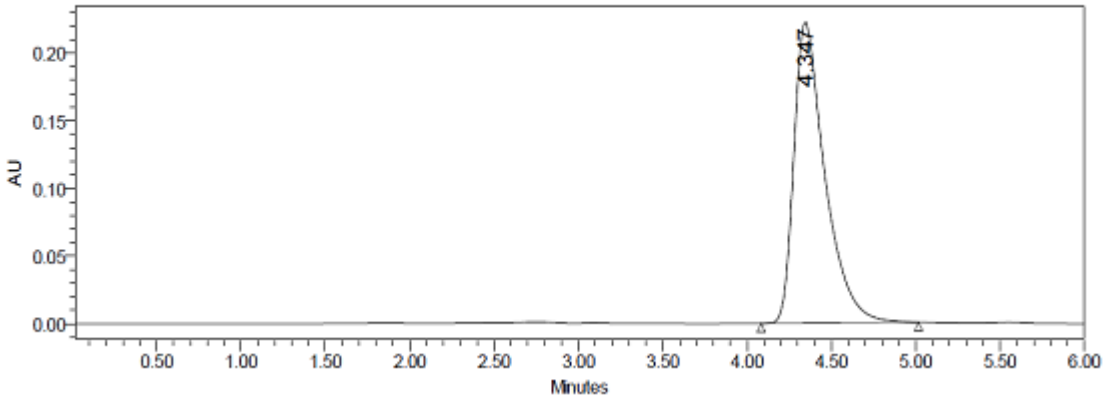
Linearity Chromatogram of 10 µg/ml



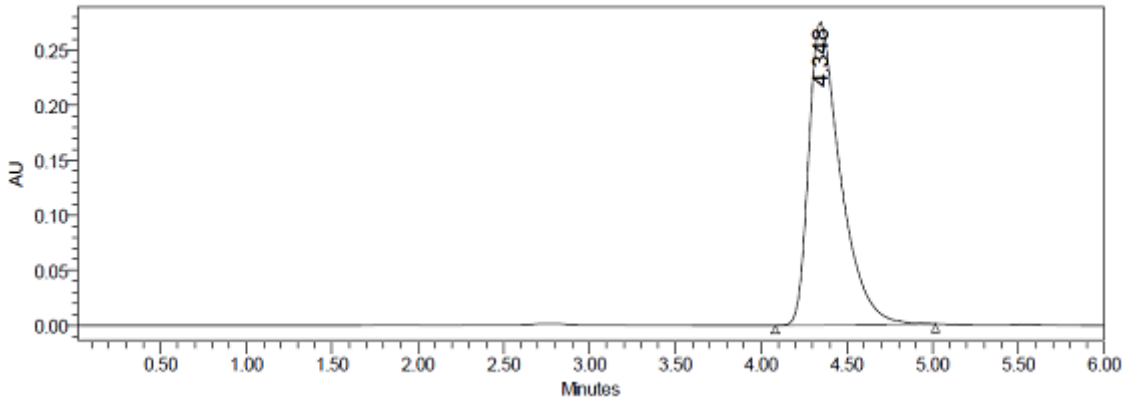
Linearity Chromatogram of 20 µg/ml



Linearity Chromatogram of 30 µg/ml



Linearity Chromatogram of 40 µg/ml

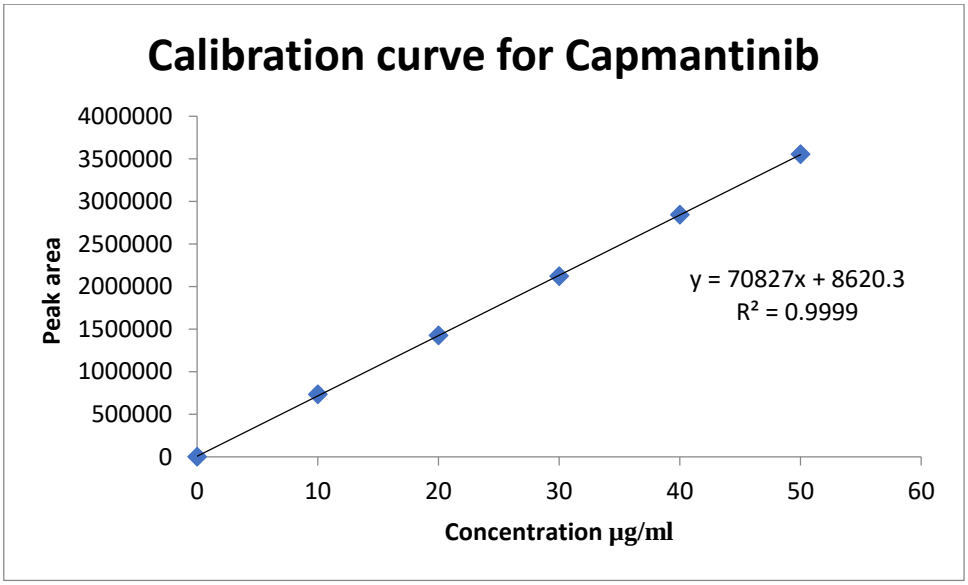


Linearity Chromatogram of 50 µg/ml

RT	Area	Height (µV)
4.350	732615	57164
4.349	1425124	111274
4.348	2122337	165927
4.347	2844280	222559
4.348	3551477	275507

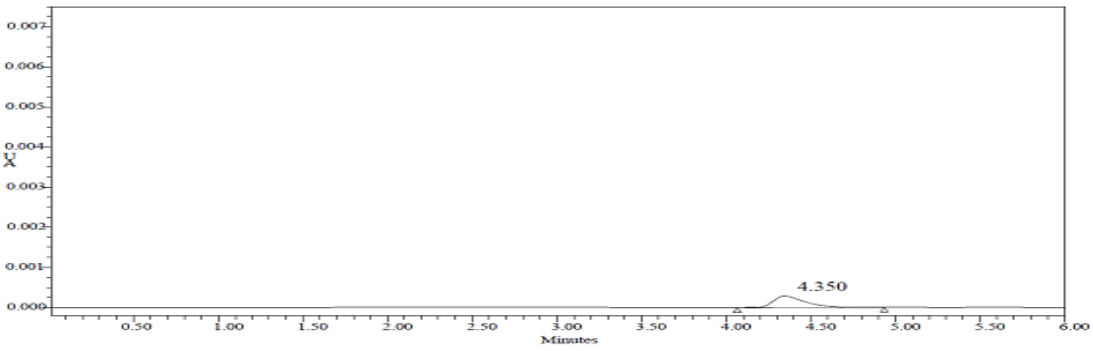
Linearity Study Data

S.No	Concentration µg/ml	Average Peak Area	Correlation Coefficient	LOD	LOQ	Slope	Intercept
1	10	732615	0.999	0.027 µg/ml	0.09 µg/ml	70827	8620
2	20	1425124					
3	30	2122337					
4	40	2844280					
5	50	3551477					



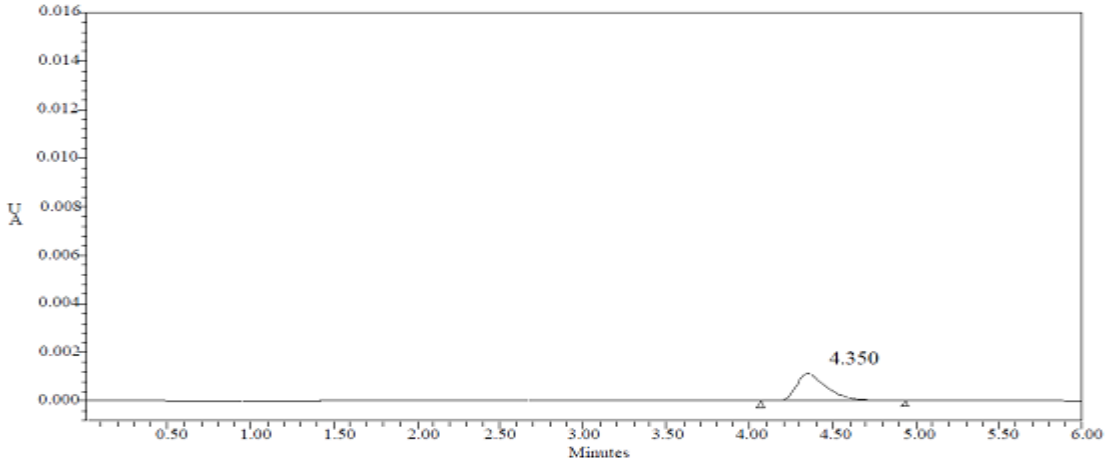
Calibration Curve

LOD & LOQ



Retention Time (min)	Area (µV*sec)	Height (µV)
4.350	1974	154

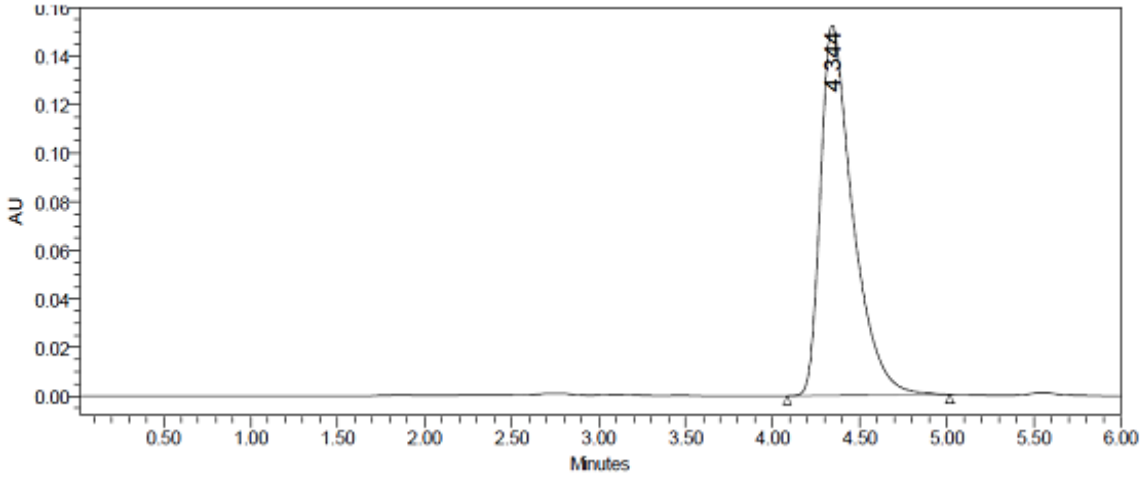
Chromatogram for LOD

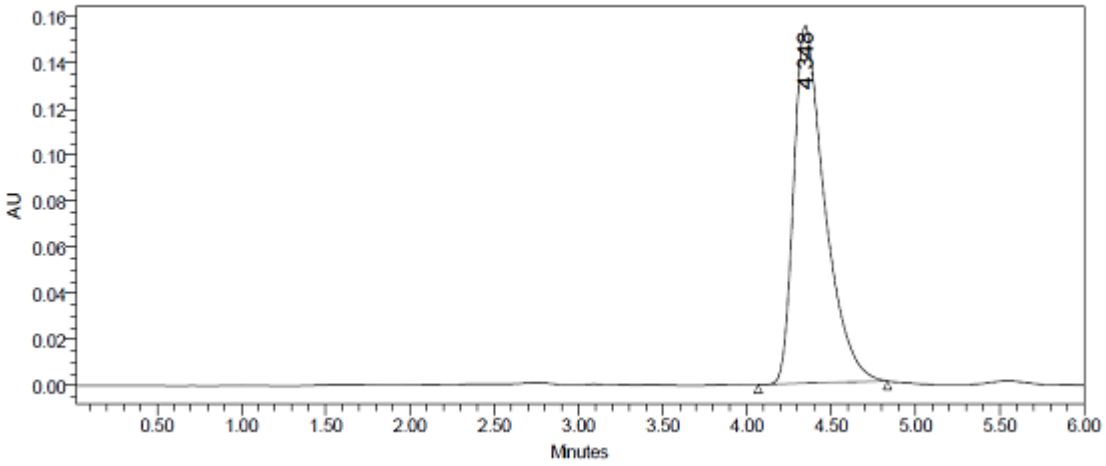


Retention Time (min)	Area (µV*sec)	Height (µV)
4.350	6613	516

Chromatogram for LOQ of Capmantinib by RP-HPLC Method

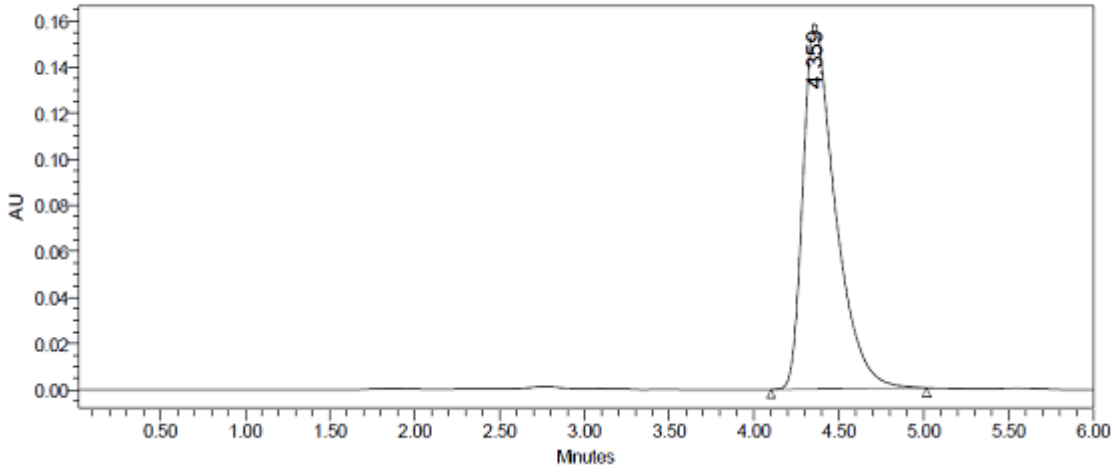
Quantification of Formulation

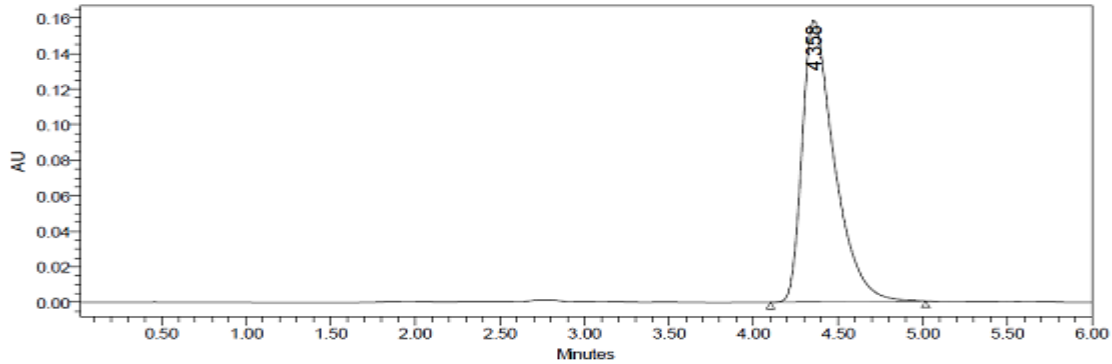




RT	Injection	Area	USP Plate Count	USP Tailing
4.344	1	1963547	2686.9	1.6
4.348	2	1985850	2664.2	1.6

Chromatogram for Assay of Capmantinib Std





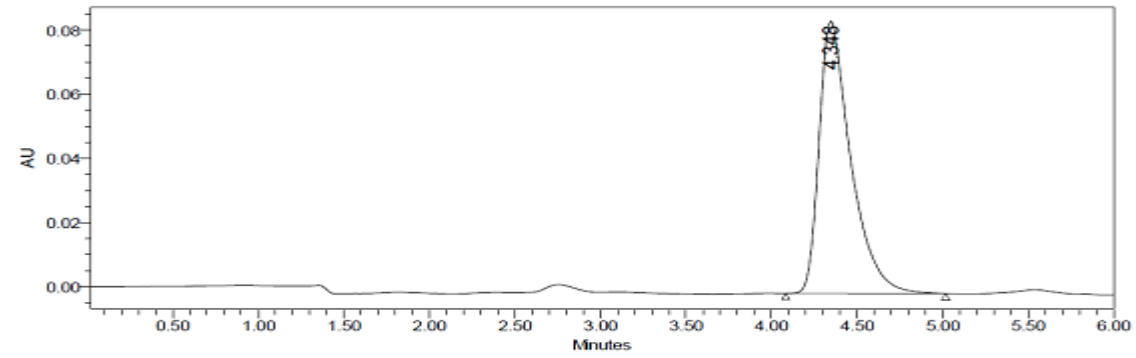
RT	Injection	Area
4.359	1	2097588
4.358	2	2097186

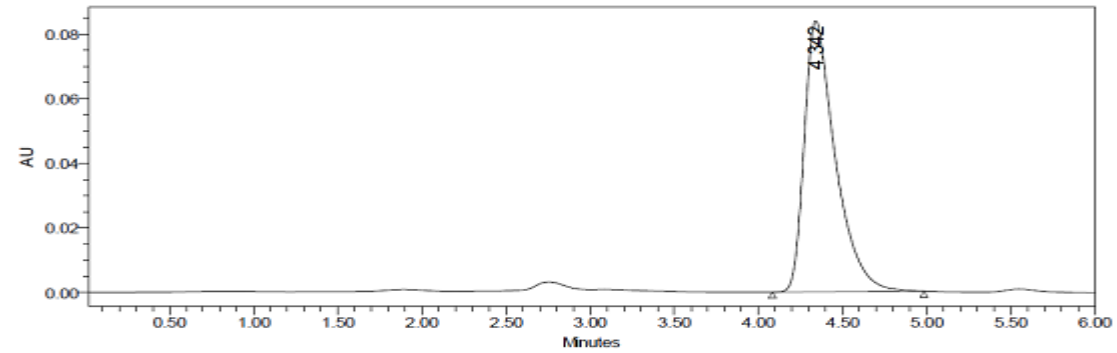
Chromatogram for Assay of Capmantinib Sample

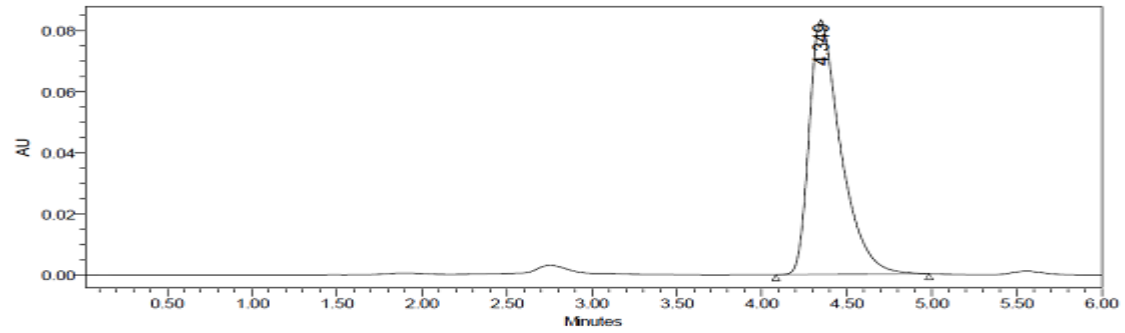
Quantification of formulation- Data

S.No	Standard Peak Area	Sample Peak area	Percentage purity (%)	Average Percentage (%)	SD	%RSD
1	1963547	2097588	101.32	100.91	0.5798	0.5745
2	1985850	2097186	100.50			

Recovery study:

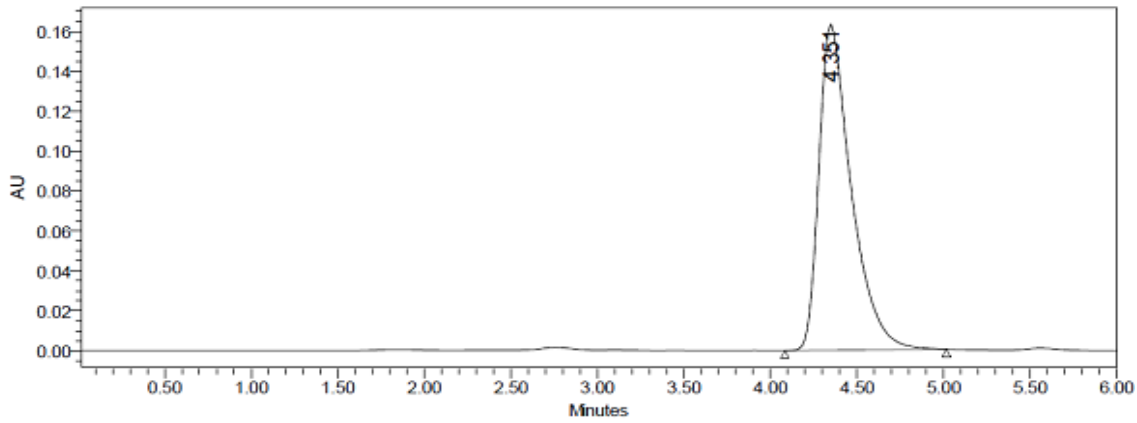


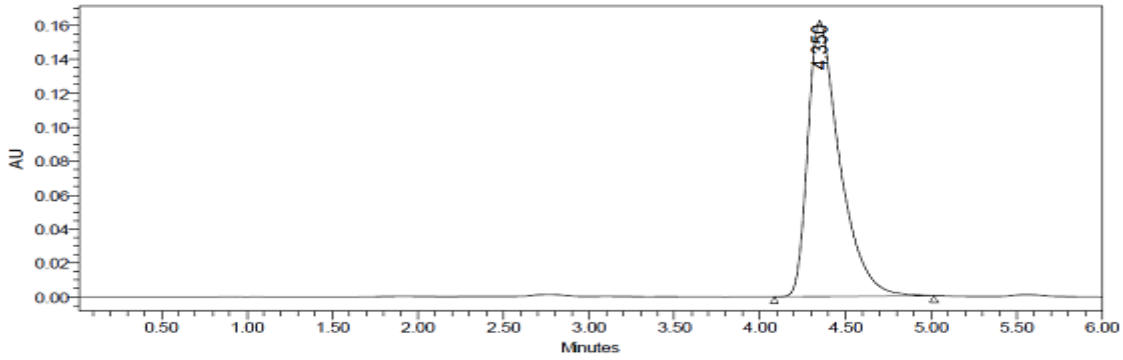


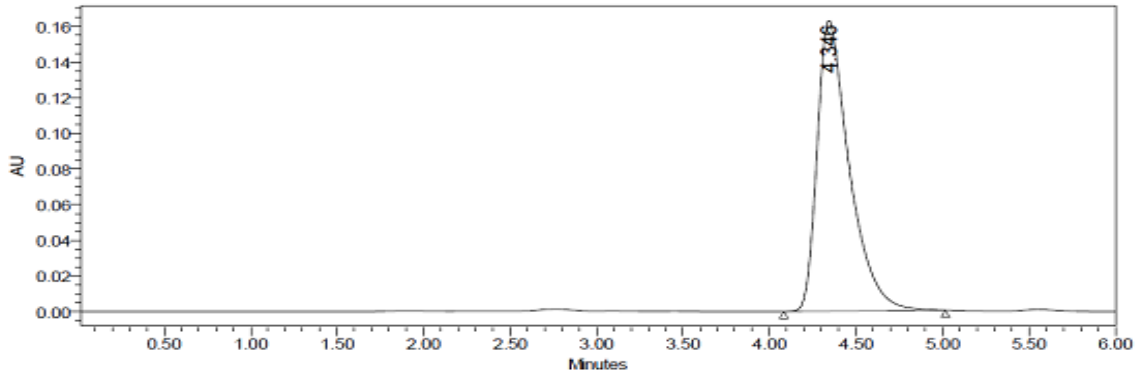


RT	Injection	Area
4.348	1	1093945
4.342	2	1080744
4.349	3	1077761

Recovery -50%

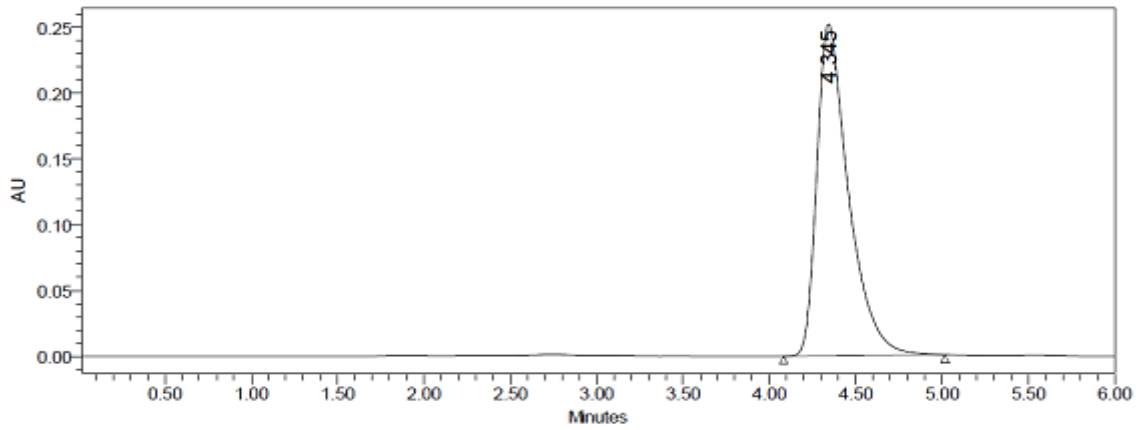


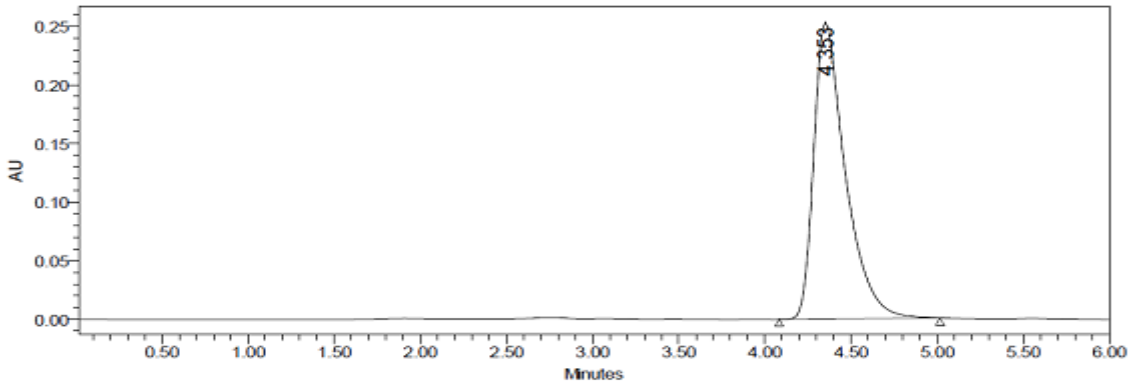


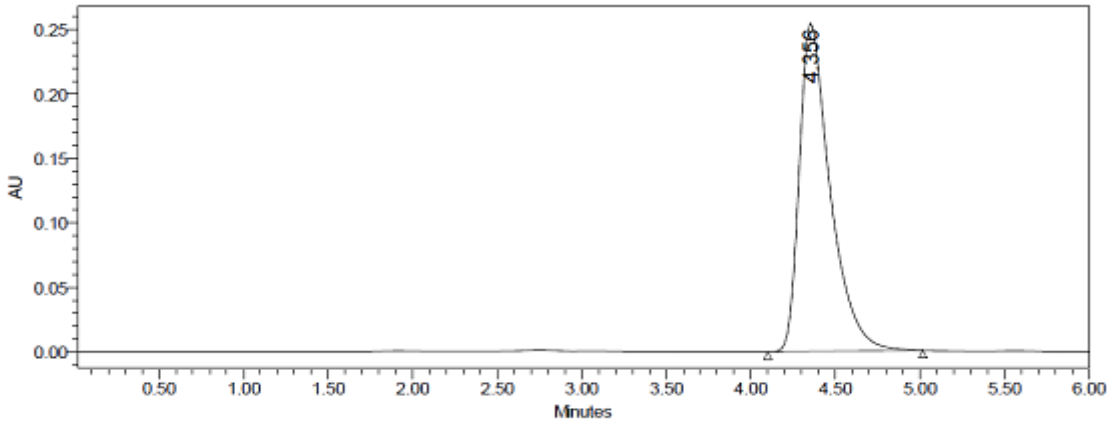


RT	Injection	Area
4.351	1	2122514
4.350	2	2103477
4.346	3	2114088

Recovery - 100%







RT	Injection	Area
4.345	1	3221451
4.353	2	3227346
4.356	3	3231256

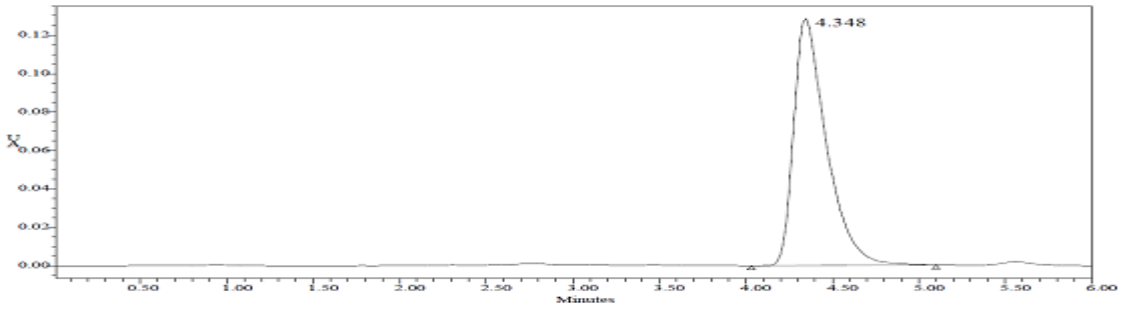
Recovery -150%

Recovery Study data

% Concentration	Average Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery	SD	% RSD
50%	1084150	5.36	5.46	101.8%	101.6%	0.2	0.19
100%	2113360	10.5	10.6	101.4%			
150%	3226684	16.0	16.2	101.6%			

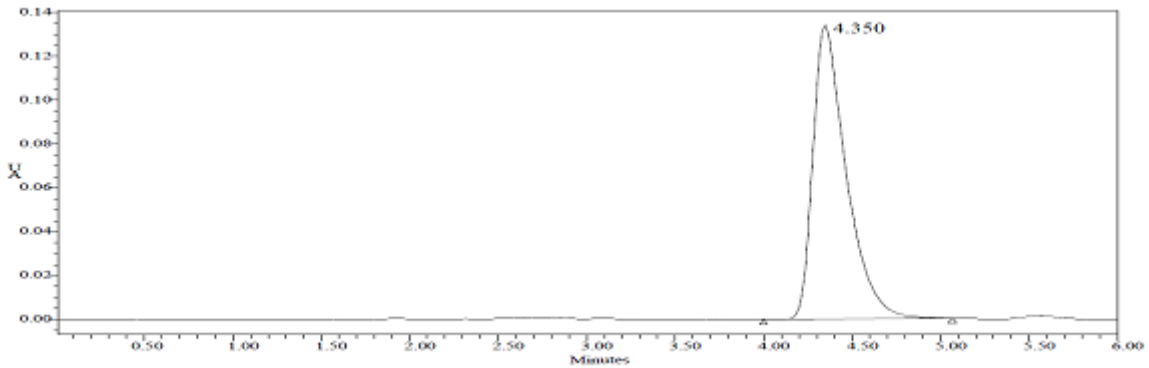
%RSD value less than 2% was found

Precision and Intermediate Precision



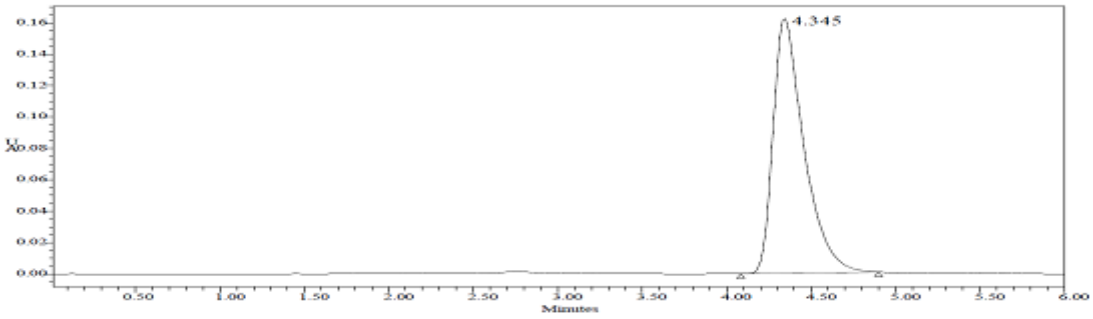
Retention Time (min)	Area (µV*sec)
4.348	1992433

Precision study (1)



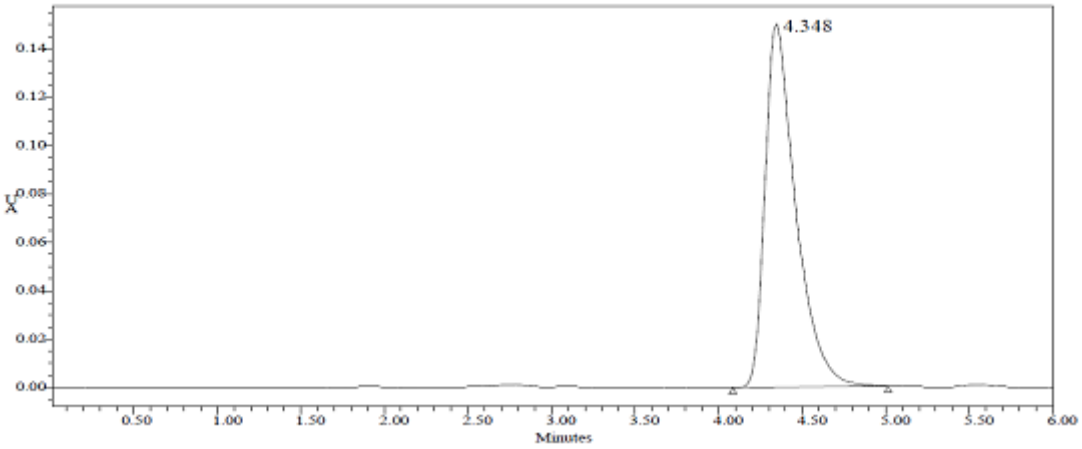
Retention Time (min)	Area (µV*sec)
4.350	1957712

Precision study (2)



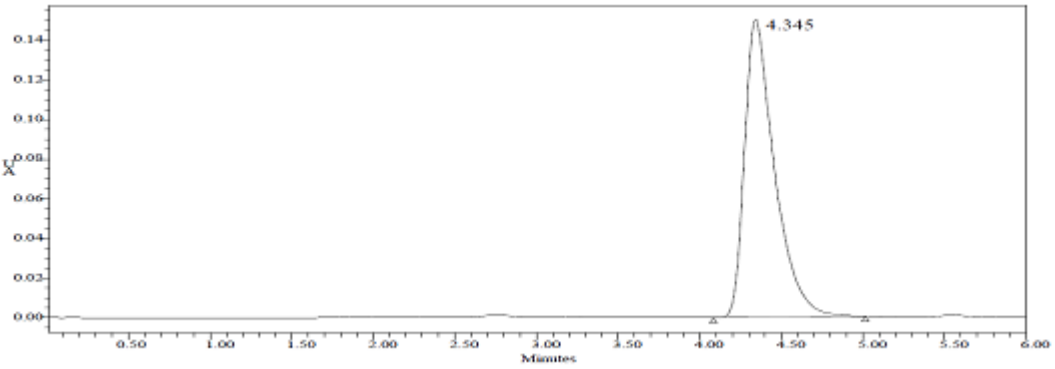
Retention Time (min)	Area (µV*sec)
4.345	1964881

Precision study (3)



Retention Time (min)	Area (µV*sec)
4.348	1923517

Precision study (4)



Retention Time (min)	Area (µV*sec)
4.345	1928095

Precision study (5)

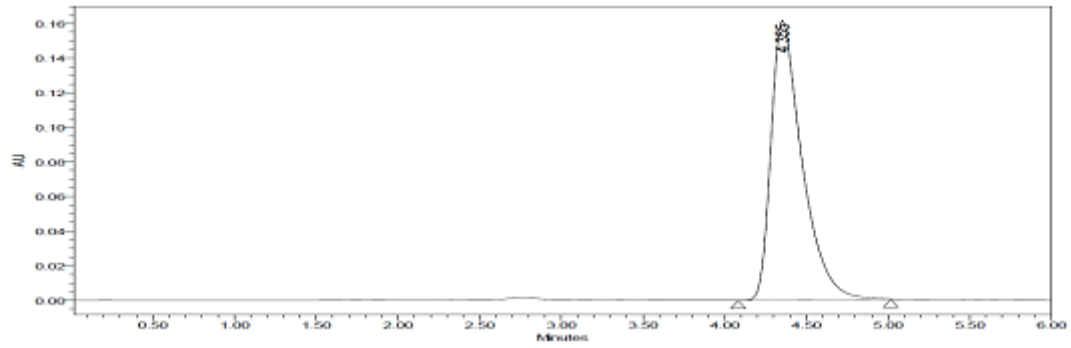
Precision Study Data

S.No	Peak Area	Average	SD	% RSD
1	1992433	1953328	28316.4	1.44
2	1957712			
3	1964881			
4	1923517			
5	1928095			

%RSD value less than 2% was found

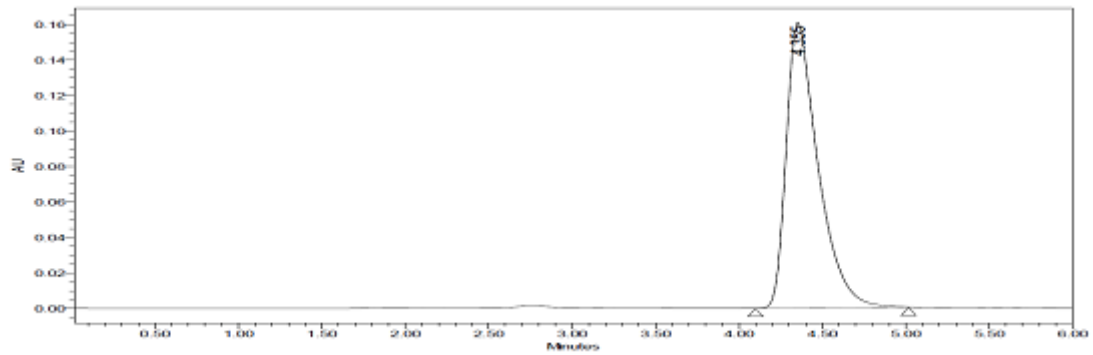
Intermediate precision:

Intermediate precision:



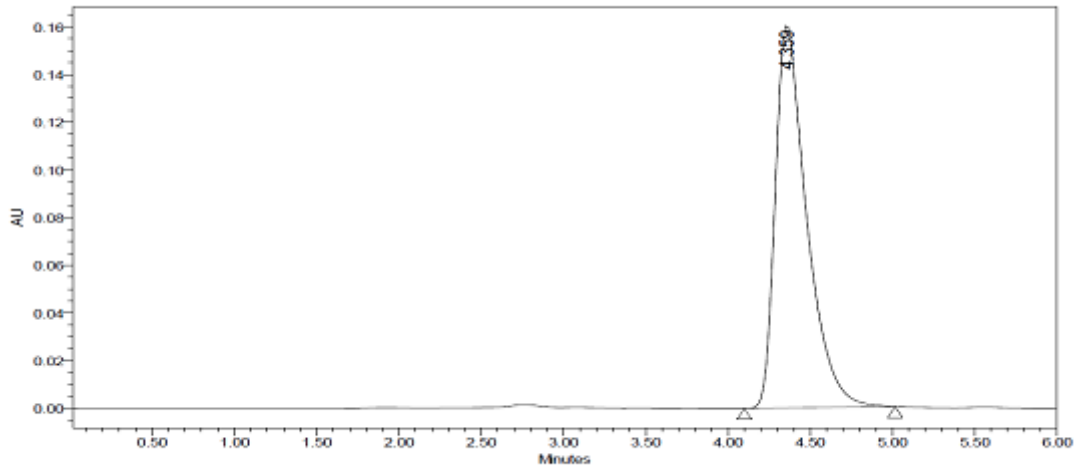
RT	Area	Height
4.355	2132155	161816

Intermediate precision Study (1)



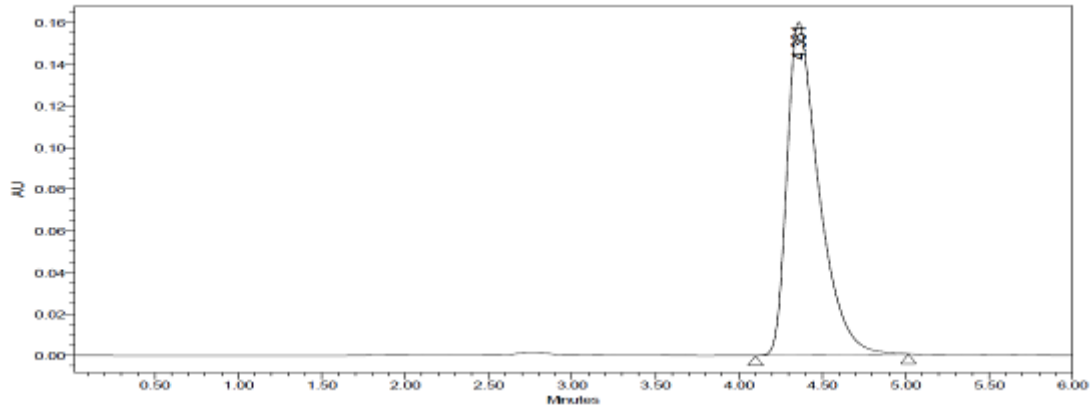
RT	Area	Height
4.355	2133191	161179

Intermediate precision Study (2)



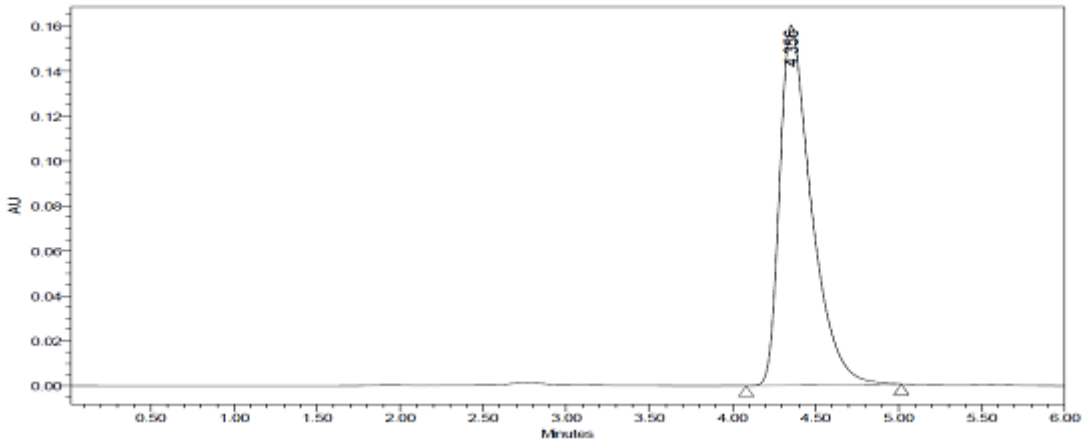
RT	Area	Height
4.359	2127358	161047

Intermediate precision Study (3)



RT	Area	Height
4.361	2123351	160105

Intermediate precision Study (4)



RT	Area	Height
4.356	2120112	160623

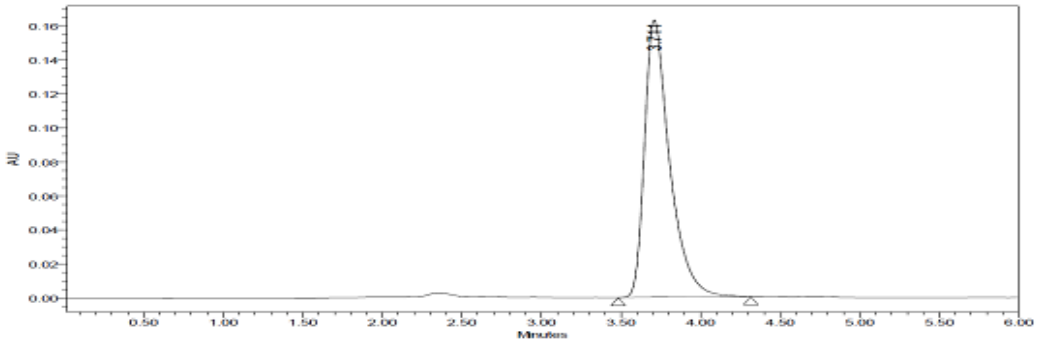
Intermediate precision Study (5)

Intermediate Precision

S.No	Peak Area	Average	SD	% RSD
1	2132155	2127233	5601.7	0.26
2	2133191			
3	2127358			
4	2123351			
5	2120112			

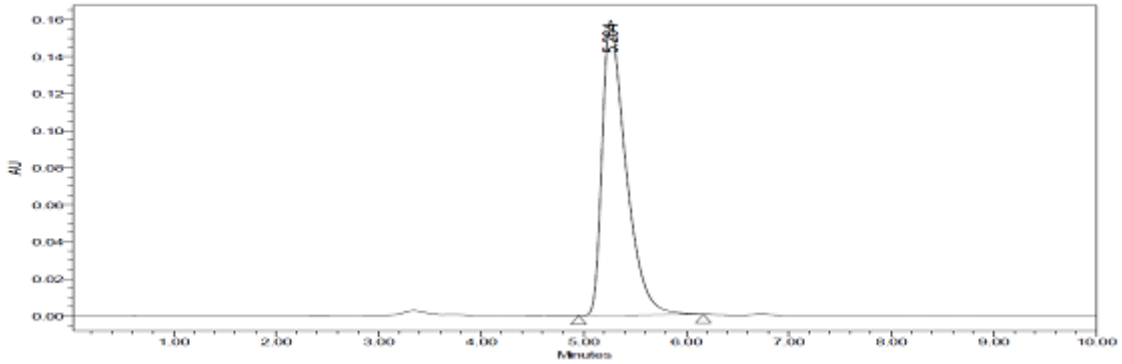
%RSD value less than 2% was found

Robustness



RT	Area	Height	USP Plate Count	USP Tailing
3.711	1789754	163567	2773.7	1.5

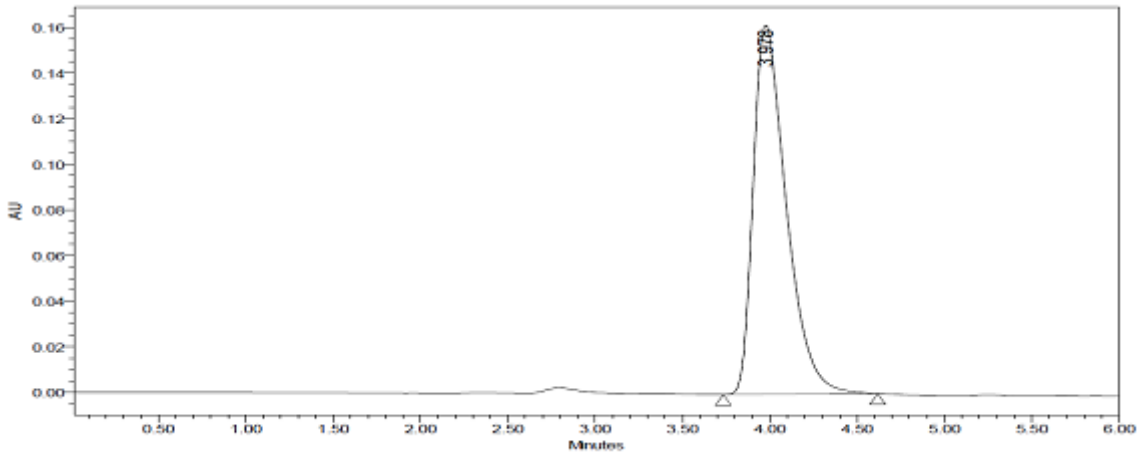
Robustness -More flow



RT	Area	Height	USP Plate Count	USP Tailing
5.264	2564130	159352	2451.7	1.7

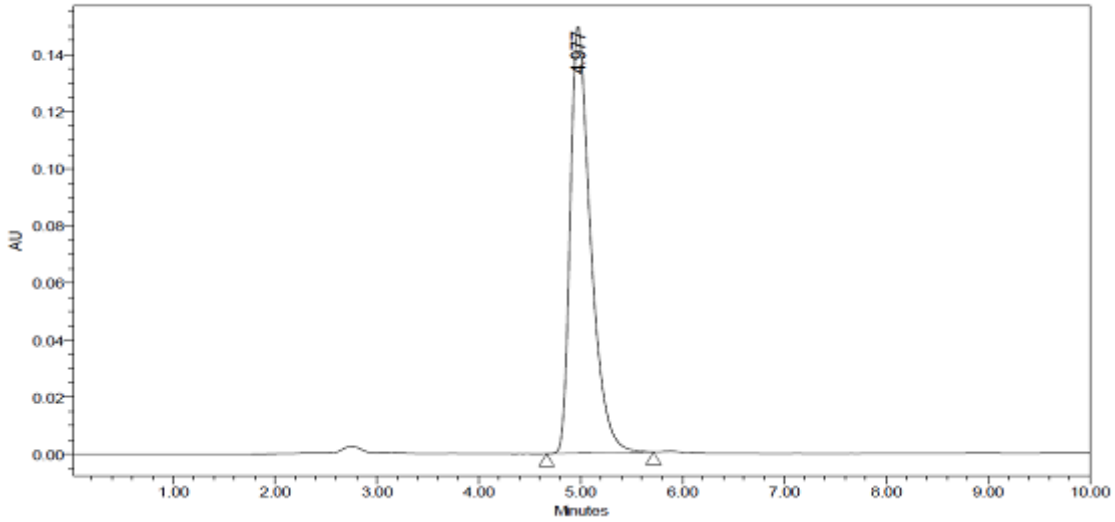
Robustness -Less flow

Robustness – Flow Rate Variation data



RT	Area	Height	USP Plate Count	USP Tailing
3.978	2128233	161853	2145.0	1.6

Robustness- More Organic



RT	Area	Height	USP Plate Count	USP Tailing
4.977	2092251	149696	2975.6	1.5

Robustness- Less Organic

Robustness – Change in Organic Composition in Mobile Phase

S.No S.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2975	1.5
2	*Actual	2669	1.6
3	10% more	2145	1.6

CONCLUSION

In the present investigation, we have developed and validated simple, sensitive, precise and accurate RP HPLC method for the quantitative estimation of Capmatinib and pharmaceutical formulations. The results expressed in Tables and figures for HPLC method are promising. The HPLC method is more sensitive, precise and accurate compared to the spectrophotometric methods. This method can be used for the routine determination of Capmatinib in bulk drug and in Pharmaceutical formulations.

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BIBLIOGRAPHY:

1) https://en.wikipedia.org/wiki/Capmatinib
2) Wiley ICH Quality Guidelines: An Implementation Guide 2018 John & Sons, Inc
3) Text book of instrumental method of chemical analysis 2018 Edition Gurdeep R. Chatwal Sham K. Anand:24-27
4) https://www.chemspider.com/Chemical-Structure.25069712.html
5) https://pubchem.ncbi.nlm.nih.gov/compound/25145656
6) Instrumental methods of analysis 2012 Edition Hobart H . Willard Merritt Dean Settle:583-592.
7) https://go.drugbank.com/drugs/DB11791
8) Text book of pharmaceutical analysis, Forth edition, Dr. S. Ravi Sankar
9) High Performance Liquid Chromatography volume 1, P.D. Sethi.