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Estimation of Crizotinib in Capsule Dosage Form by RP-HPLC

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Abstract: *A simple, rapid, precise, sensitive and reproducible high performance liquid chromatographic method was developed for the estimation of Crizotinib in capsule dosage form. Chromatographic separation of the drug was achieved on a Syncronis C-18 (250 × 4.6 mm, 5µ) column, mobile phase comprising a mixture of methanol and buffer (60:40v/v) at a flow rate of 1 ml/min. The drug eluted was monitored at 267 nm, retention time, 4.15 min. & calibration curve was linear over the range of 10 µg-1000 µg/ml. The performance of the method was validated according to ICH guidelines. The method can be applied for determination of drug in its capsule dosage form without any interference from excipients. The proposed method is suitable for routine quality control analysis.*

Keywords: *Crizotinib, RP-HPLC, Validation.*

I. Introduction

Crizotinib is an inhibitor of receptor tyrosine kinases, including ALK, Hepatocyte Growth Factor Receptor (HGFR, c-Met), RON. Translocations can affect the ALK gene, resulting in the expression of oncogenic fusion proteins. The formation of ALK fusion proteins results in activation and dysregulation of the gene's expression and signaling which can contribute to increased cell proliferation and survival in tumors expressing these proteins. Crizotinib is used to treat certain types of lung cancer.Literature survey reveals that few HPLC Methods have been reported for the estimation of titled drug in plasma [1-4]. Our aim is to develop a new, simple HPLC method for the estimation of Crizotinib in capsule dosage form.

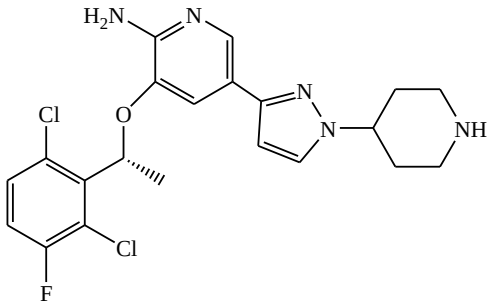

Fig. 1-Chemical structure of Crizotinib

II. Materials And Methods

Drugs, Chemicals and Solvents

Pure drug of Crizotinib was purchased from Apexbio Technology LLC, United States. Crizotinib capsule - 250mg, was purchased from local market, methanol [HPLC grade – Merck, Hyd], Potassium dihydrogen phosphate (AR grade – Finar, Hyd) were used in the preparation of mobile phase. Double distilled water (in house) was used throughout the analysis. Mobile phase was filtered using 0.45 µm membrane filter (Millipore – Millipore Pvt Ltd, Bangalore, India).

Instrumentation

The RP-HPLC analysis was accomplished on SHIMADZU High Pressure Liquid Chromatography, equipped with pump (LC-20AD), manually operating Rheodyne injector with 20µl sample loop, Syncronis (25cm×4.6mm) analytical column containing C-18 silica gel, 5µ size. All the parameters of HPLC were controlled by LC SOLUTION software; other instruments used were double beam UV-Visible Spectrophometer (Elico-210), electronic balance (Shimadzu–Aux 220), digital pH meter (Systronics) and Bio-technics ultrasonic bath Sonicator.

Selecton of detection wavelength

The drug was dissolved in methanol (100µg/ml), and scanned in the UV range, from the spectrum (Figure: 2), 267 nm was selected as a detection wavelength.

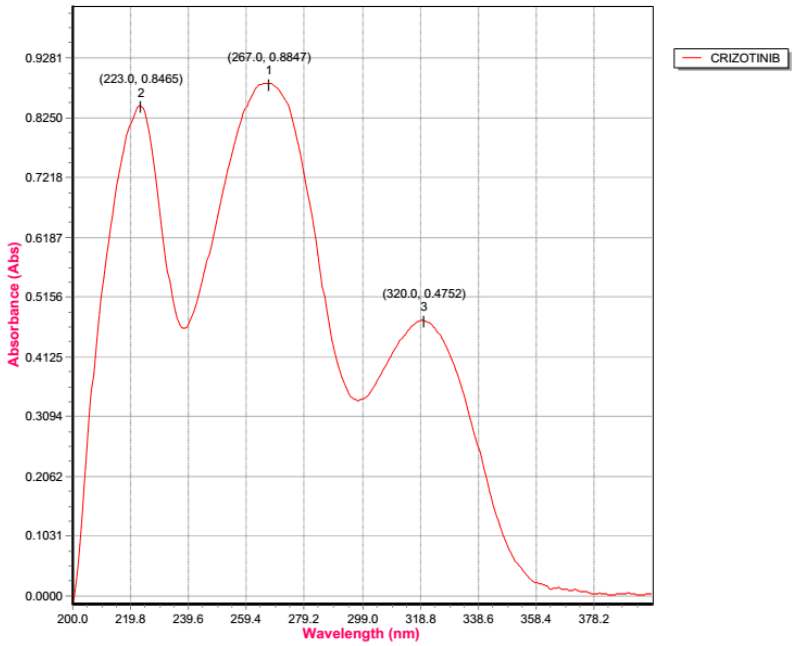

Fig.2-UV spectrum of Crizotinib

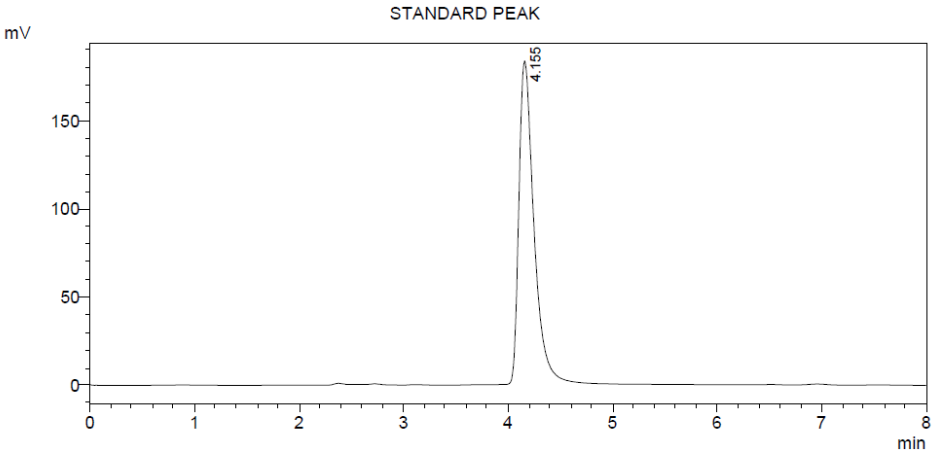

Fig-3: Standard chromatogram of Crizotinib

Table-1: Optimized Conditions for the Proposed HPLC Method

Stationary Phase	Syncronis C-18 (250×4.6mm,5µ) column
Mobile Phase	Methanol: Buffer(0.3% w/v KH_2PO_4, pH 3) (60:40 v/v)
Flow rate	1 ml/min
Detection wavelength	267nm
Injection Volume	20µl
Run time	8 min

Method Development and Optimization

To develop a suitable HPLC method for the determination of Crizotinib, trials were performed with different mobile phases, using methanol, acetonitrile, with water, buffers at different pH values, with varying compositions of mobile phases like 50:50 (methanol: water), 50:50 (Acetonitrile: water), 70:30 (methanol: 0.3% Di potassium hydrogen phosphate buffer, pH-7), 40:60 (methanol: Potassium dihydrogen phosphate buffer , pH-3), 50:50 (methanol: 0.3% potassium dihydrogen phosphate, pH-3), 55:45 (methanol : buffer, pH-3). The method was optimized finally using combination of methanoland KH_2PO_4 buffer (pH - 3) in the ratio of 60:40 v/v with a flow rate of 1ml/min. The drug was eluted at retention time of 4.15 min, and monitored at 267 nm. The run time was set for 8 minutes. All the determinations were performed at isocratic elution mode and the injection volume - 20µl.

Preparation of the Mobile Phase

The mobile phase was prepared by mixing of methanol and 0.3% w/v Potassium dihydrogen orthophosphate (pH-3 adjusted with 1N OPA) in the ratio of 60:40 v/v. It was filtered through 0.45µ filter paper then degassed by sonication.

Solvent: Methanol was used as a solvent to dissolve the drug.

Diluent: Mobile phase.

Preparation of standard stock solution

10 mg of Crizotinib was weighed accurately and transferred into 10 ml volumetric flask, about 5 ml of methanol was added to dissolve the drug and then the volume was made up to the mark with the same, to give a concentration of 1mg/ml standard stock solution.

Preparation of working standard solutions

Working standard solutions having concentrations, 1µg/ml, 10, 50, 100, 200, 300, 400 up to 1000µg/ml were prepared by appropriate dilutions of the standard stock solution with the diluent. The solutions thus prepared were filtered through 0.45µ membrane filter and sonicated for 5 min.

Preparation of Test solution:

20 capsules were emptied, weighed, mixed thoroughly, test sample equivalent to 25 mg of Crizotinib (Capsule formulation: 250mg as per the label claim) was accurately weighed transferred into 50 ml beaker, about 15 ml of methanol was added to dissolve the drug, the solution was first, filtered through Whatmann filter paper, washed the residue and filter paper with methanol and collected into the same volumetric flask (25 ml), to extract the drug as completely as possible and made up to the mark with the same, then after filtered through 0.45µm membrane filter (Stock solution). From this 3ml of solution was pipetted out and transferred into 10 ml of volumetric flask and the volume was made up with diluent to give concentration of 300 µg/ml, as per the label claim.

Assay

Standard (300µg/ml) and test sample solutions were injected under identical chromatographic conditions as mentioned earlier and chromatograms were recorded. This was done in triplicate. The obtained mean test peek area was compared with standard mean peak area and the amount was calculated.

$$\%\ \text{Drug Content} = \frac{\text{Test Area}}{\text{Standard Area}} \times \text{Standard Conc} \times \frac{\text{Avg . wt}}{\text{sample wt}} \times \frac{100}{\text{Label claim}}$$

Table 9: Results of Assay

S.NO	Parameter	Crizotinib
1	Label claim	250mg
2	Standard peak area	1434473
3	Test peak area	1432081
4	Sample weight	32mg
5	Average weight	320mg
6	Amount obtained	248.7
7	**% Assay**	**99.48**

Method Validation

The developed method was validated as per ICH guidelines [5, 6] with respect to parameters such as linearity, range, precision, accuracy, robustness, specificity, system suitability and solution stability.

Linearity

Working standard solutions in the range of 1µg/ml to 1mg/ml were prepared by appropriate dilutions of the standard stock solution and 20µl of each of standard solutions were injected at the optimized chromatographic conditions and the chromatograms were recorded. The peak areas were recorded. Calibration curve was constructed by plotting concentrations on X-axis, peak areas on Y- axis and regression equation was computed.

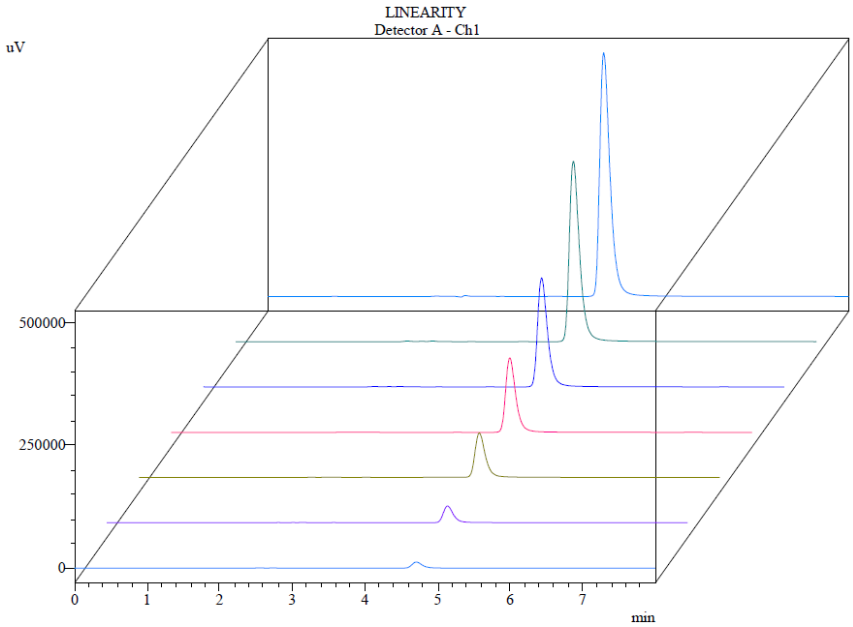

Fig.4-Overlay Chromatogram of linearity

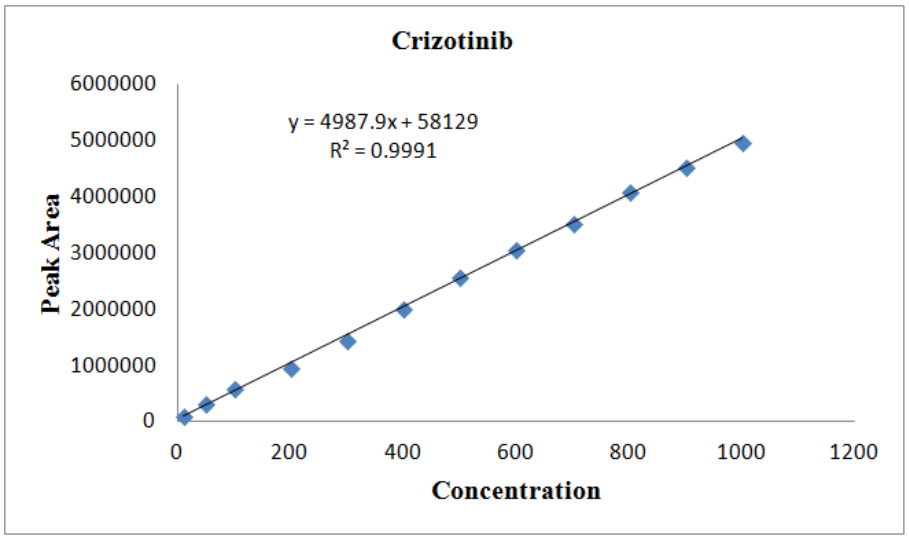

Fig.5-Calibration curve of crizotinib

Table 2: Results of Linearity

S. No.	**Concentration (µg/ml)**	**Peak area**
1	10	123931
2	50	329121
3	100	619666
4	200	985027
5	300	1454473
6	400	2027706
7	500	2599095
8	600	3081330
9	700	3555841
10	800	4098756
11	900	4563210
12	1000	4992264
Statistical Analysis	**Slope**	**4987**
	Correlation Coefficient(r^2)	**0.999**
	Y-Intercept	**58129**

Range

Working standard solutions, 10µg/ml (lower concentration) and 1000µg/ml (higher concentration) were prepared by appropriate dilution of the standard stock solution with the mobile phase. The prepared solutions were filtered through 0.45µ membrane filter and sonicated for 5min. 20 µl of each standard solution was injected six times into the chromatographic system, peak areas were measured and % RSD calculated.

Table 3: Results of Range

S. NO	Peak area	
	10 µg/ml	**1000 µg/ml**
1	732696	3158973
2	753954	3101889
3	732362	3168105
4	738608	3147428
5	737073	3113993
6	734528	3114455
Avg	**738203.5**	**3134141**
SD	**8093.02**	**27496.9**
%RSD	**1.09**	**0.87**

System suitability

System suitability test was performed by injecting standard solution of Crizotinib (250 µg/ml) into the stabilized HPLC system, six times. The system suitability was established by evaluating repeatability, tailing factor (T) and theoretical plates (N), Resolution (R) from the standard chromatograms obtained.

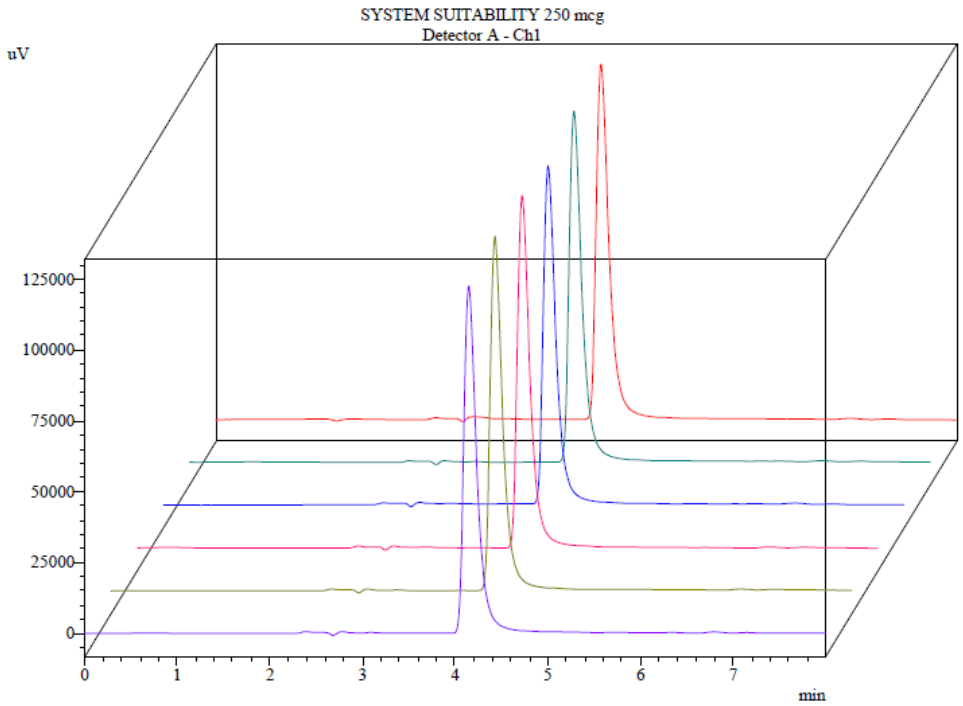

Fig.5- Overlain Chromatogram of system suitability

Table 4: System suitability data

Injection	Peak area
1	1186638
2	1193515
3	1174090
4	1182037
5	1181879
6	1178347
Avg	**1182751**
SD	**6732.767**
%RSD	**0.56**

Precision

System precision was performed by injecting; six replicate injections of standard solution (355µg/ml) and the chromatograms were reviewed for the % RSD of peak areas. Method precision was demonstrated by preparing six test solutions at 250µg/ml concentrations as per the test procedure and recording the chromatograms. The % RSD for assay of six samples was calculated. Intermediate precision of the analytical method was determined by performing method precision on different days and by different analysts under same experimental conditions.

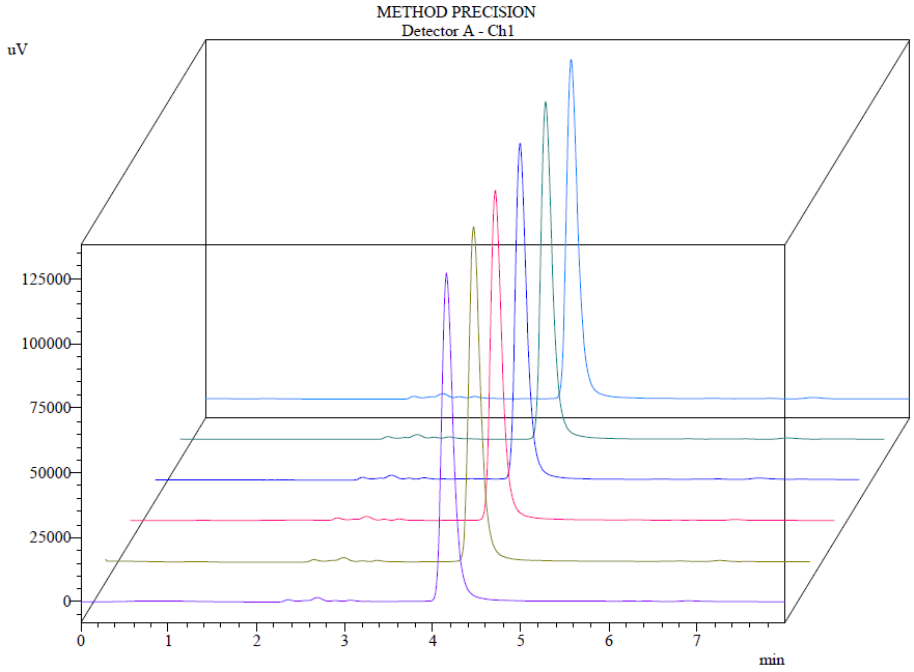

Fig.6- Overlay Chromatogram of Method Precision

Table 5: Method Precision Data

S.NO	Peak area
1	1164845
2	1178531
3	1156323
4	1184852
5	1180649
6	1175732
Avg	**1173489**
SD	**10772.43**
%RSD	**0.91**

Table 6: Intermediate precision data (Day 1 and 2)

Drug name	DAY 1 Peak area	%Assay	DAY 2 Peak area	%Assay	%Pooled RSD
	1078514	99.09	1076418	98.91	
	1078213	99.06	1055542	97.09	
Crizotinib	1086732	99.80	1053554	96.91	**1.68**
	1076341	98.90	1064784	97.89	
	1113798	102.1	1051535	96.74	
	1095748	100.56	1052313	96.80	

Table 7: Intermediate precision data (analyst 1 and analyst 2)

Drug name	Analyst 1 Peak area	%Assay	Analyst 2 Peak area	%Assay	%Pooled RSD
	503751	97.96	503049	99.59	
	512394	99.47	505332	98.24	
Crizotinib	504797	98.14	507069	98.54	**0.68**
	504802	98.14	501066	97.50	
	499685	97.26	505058	98.20	
	505853	98.33	505503	98.27	

Accuracy

Accuracy of the method was ascertained on the basis of recovery studies by standard addition method. Recovery studies were carried out at three different levels (80%, 100% and 120%) by the addition of standard drug to pre-analyzed sample solution having the concentrations of 40µg/ml, 50µg/ml and 60µg/ml of Crizotinib. Triplicate determinations were carried out at each level. Mean percentage recovery values at three different levels of the drugs were calculated.

$$\%\text{Recovery} = \frac{\text{Spiked concentration} - \text{Test concentration}}{\text{standard concentration}} \times 100$$

Preparation of Standard and Test solutions

Working standard solutions containing 40µg/ml, 50µg/ml & 60µg/ml of Crizotinib were prepared in triplicate from standard stock solution by appropriate dilution. Test solution containing 70µg/ml of Crizotinib, was prepared from test stock solution.

Preparation of Spiking Solution

Spiking at 80% level was accomplished in triplicate, 0.4ml of standard stock solution was taken in a 10ml volumetric flask, to this 0.7ml of test stock solution was added, shaken and filtered through a Whatmann filter paper, the residue and filter paper washed with a diluent, filtrate and washings were collected into volumetric flask then made up to the mark. In the similar manner spiking at 100% and 120% were carried out.

Table 8: Accuracy Study Data

Level of spiking	Stdconc (µg/ml)	Peak area	Test peak area	Spiked peak area	Mean %recovery
		145682		438155	100.34
80	40	144489	291969	437208	100.51
		145910		436831	99.28
		192103		485281	100.22
100	50	192809	292751	484450	99.42
		191505		485174	100.47
		240523		534780	100.05
120	60	240975	294129	534513	99.75
		241082		534617	99.75

Specificity

Specificity was established by injecting, blank, standard and test solutions into the HPLC system with optimized chromatographic conditions.

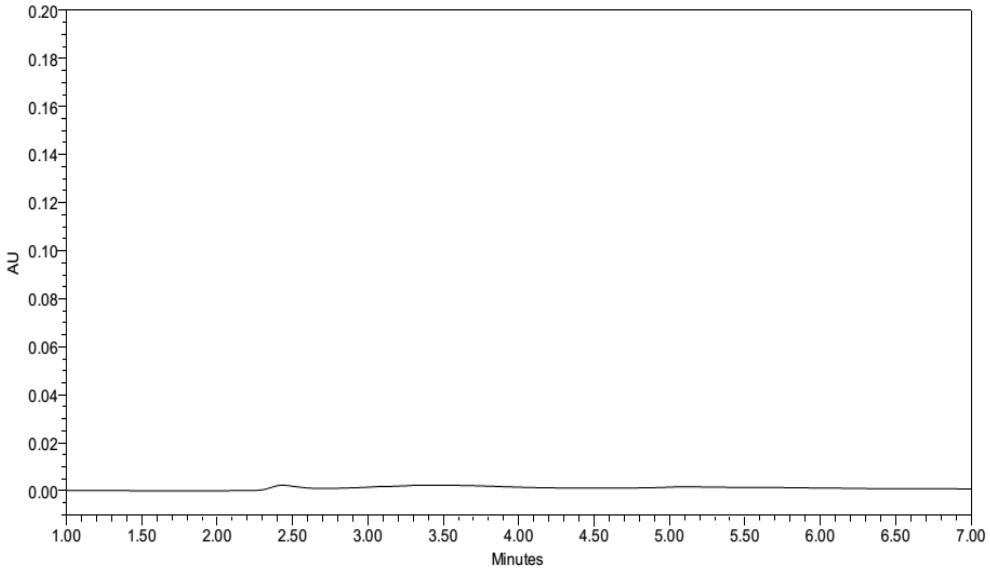

Fig.7- Blank Chromatogram

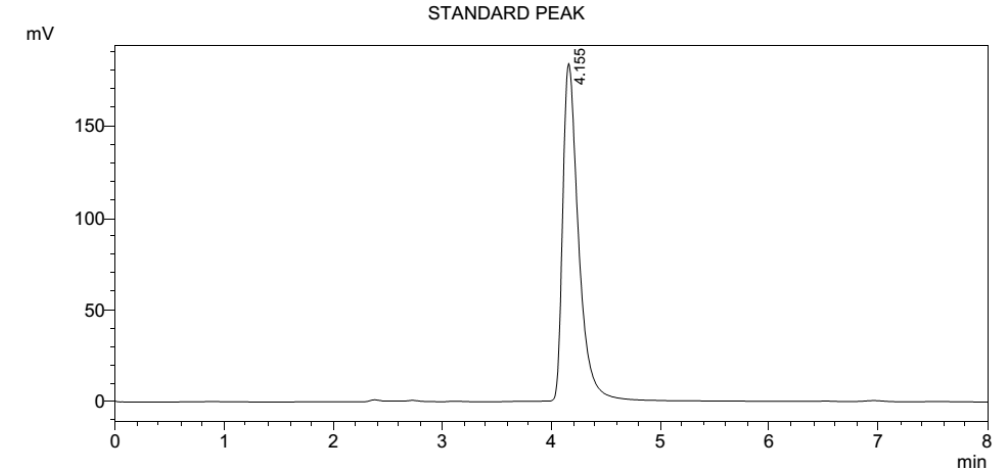

Fig.8- Chromatogram of Standard solution

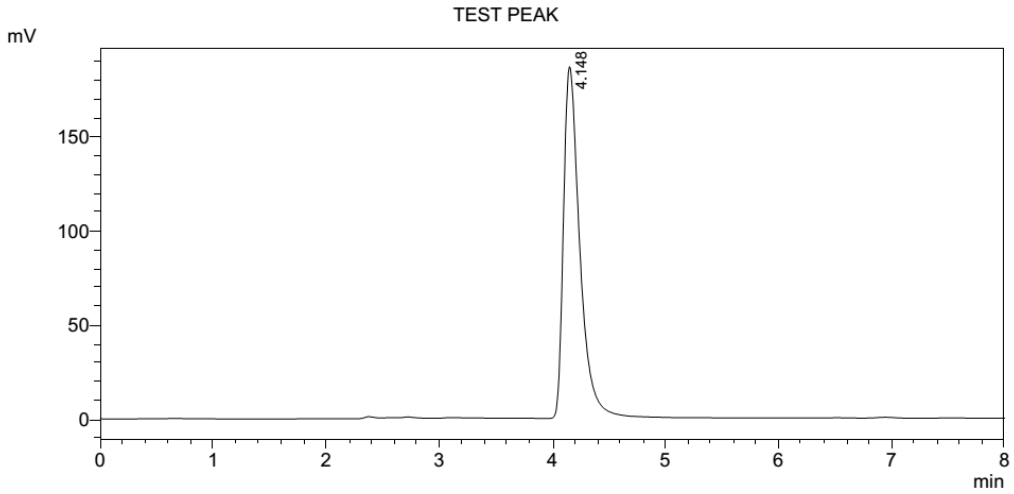

Fig.9- Chromatogram of Test solution

Robustness

Robustness of the method was demonstrated by making deliberate changes in the optimized chromatographic conditions of the developed method. Six replicate injections were given and the effects of the variations were observed in the recorded chromatograms and the %RSD of the peak areas and system suitability parameters were calculated, at each of the following conditions, variation in flow rate (±0.1ml/min), mobile phase ratio (±2%) and detection wavelength (±3nm).

Table 10: Robustness Study Data

Parameter	**Conc (525µg/ml)**	**Mean peak area**	**%RSD**
	58:42(-2%)	2067382	0.73
Mobile phase	60:40	2118286	0.29
	62:38(+2%)	2154100	1.07
	0.9ml/min(-0.1)	2543497	1.2
Flow rate (ml/min)	1ml/min	2015759	0.79
	1.1ml/min(+0.1)	1854992	0.97
	264nm(-3)	2046543	1.29
Wavelength(nm)	267nm	2064599	0.84
	270nm(+3)	1829843	0.98

Solution Stability

The standard solution (200µg/ml) and the test sample solutions (200µg/ml) of Crizotinib were prepared as per the test procedure and then injected into the RP- HPLC. Chromatograms were recorded and peak responses were measured. The same procedure was repeated at an interval of 24 hours until there was a significant change in the concentration of drugs, by comparing with freshly prepared solutions.

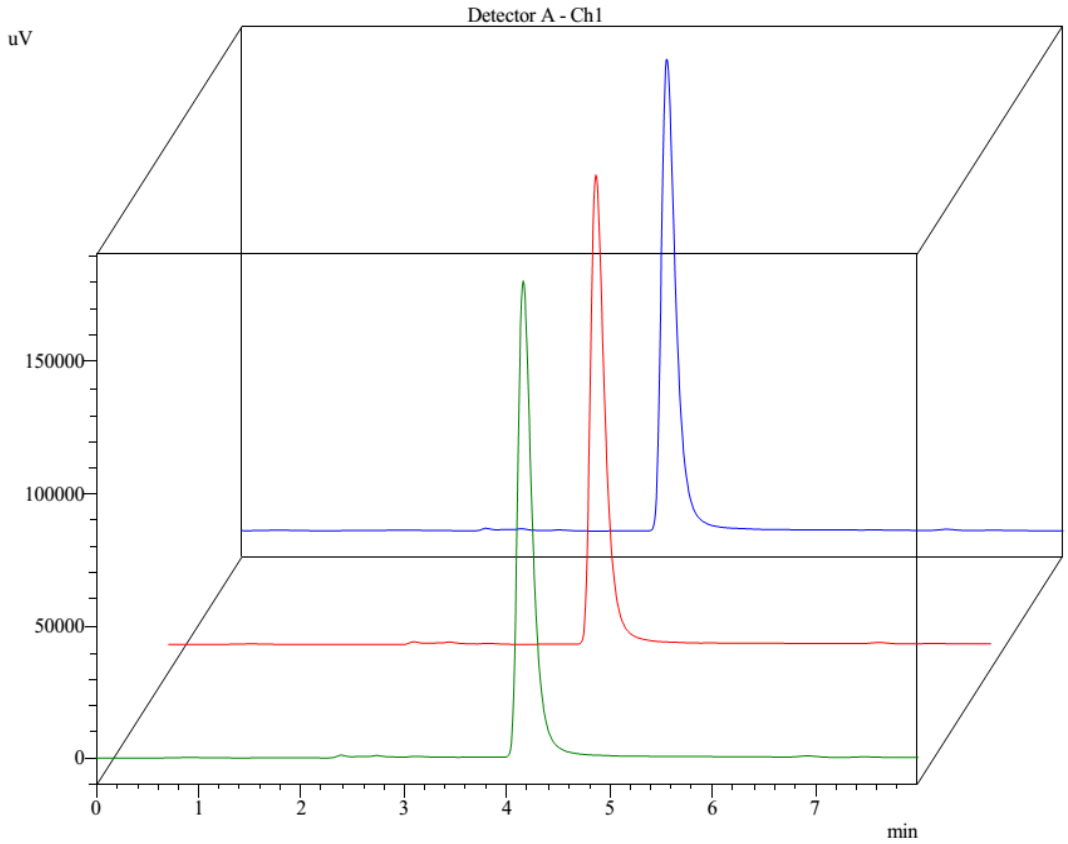

Fig.10- Chromatogram for Stability study of Standard Solution

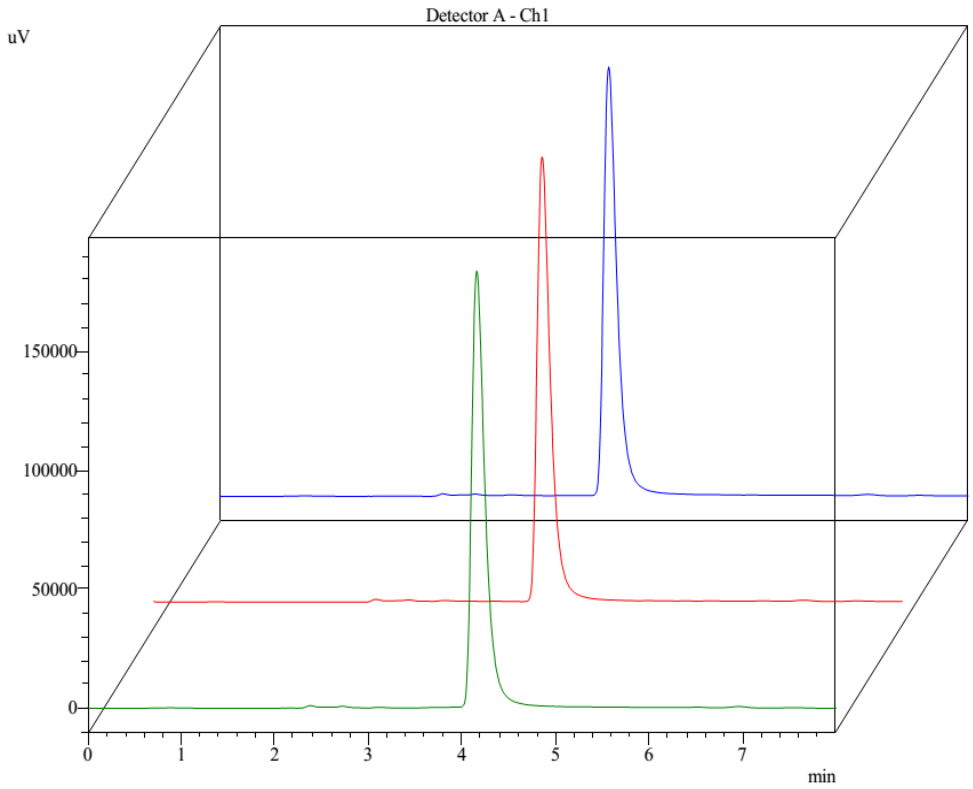

Fig.11-Chromatogram for Stability study of Test Solution

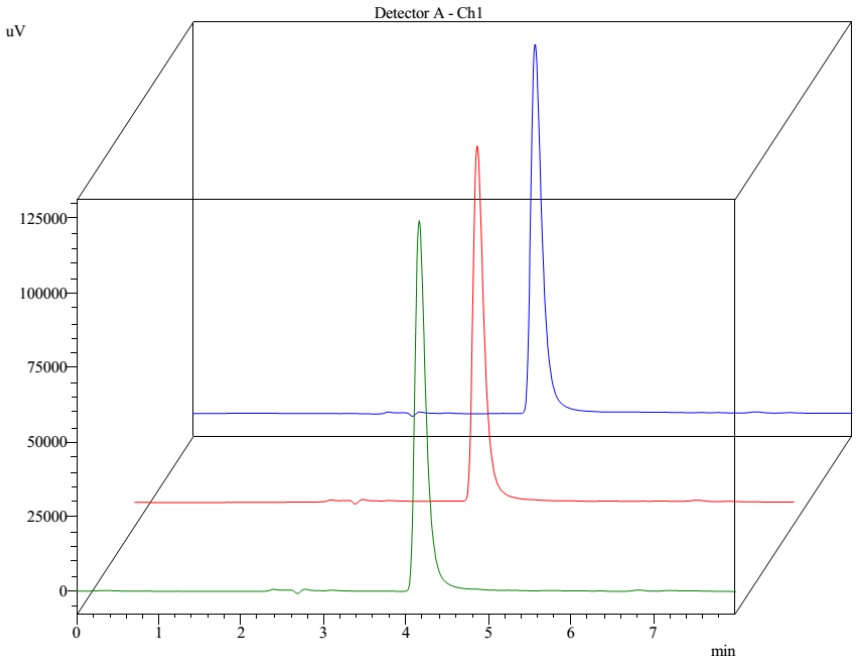

Fig.12- Chromatogram for Stability study of Mobile phase

Table 11: Standard solution Stability Study Data

Time (hours)	**Standard peak area**	**%Assay**
Intial	934862	99.55
24	925027	98.57
48	920812	98.14

Table 12: Test solution Stability Study Data

Time (hours)	**Test peak area**	**%Assay**
Intial	934531	99.52
24	924812	98.55
48	920035	98.07

III. Results And Discussion

Initially, various mobile phase compositions were tried to develop a simple HPLC method, optimum results achieved with a mobile phase, consisting a mixture of methanol and 0.3% potassium dihydrogen phosphate (pH-3 adjusted with 1N OPA) in the ratio of 60:40 v/v, delivered at a flow rate of 1ml/min. wavelength for detection - 267nm and run time - 8 min. Mobile phase used as a diluent. Crizotinib was eluted at a retention time of 4.15min. The % assay was found to be 99.48% (Table: 2). Calibration curve was linear over the range of 10-1000µg/ml (r^{2-} 0.999) (Fig: 4, 5, Table: 3, 4). From the system suitability study it was observed that all the parameters were within the limits (Fig: 6, Table: 5). Precision (Fig: 7, Table: 6-8) and accuracy (Table: 9) results were found to be within the limits. Robustness study revealed that the system suitability parameters were within the limits, even though small deliberate changes were done in method parameters (Table: 10). Standard, test solution and mobile phase were found to be stable for 2 days, (Fig: 8-10, Table: 11-12). The performance of the method was validated according to ICH guidelines.

IV. Conclusion

The proposed method was found to be simple, rapid, precise, robust, accurate and specific for the determination of Crizotinib in capsule dosage form. The sample recoveries from the formulation were in good agreement with the label claim, which suggests that the developed method was accurate. The method can be applied for the estimation of titled drug in formulation.

Acknowledgements

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