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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ASSAY OF CRIZOTINIB IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

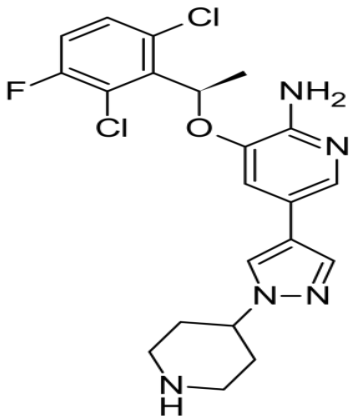
A simple, Precised, Accurate method was developed for the estimation of Crizotinib by RP-HPLC technique. Chromatographic conditions used are stationary phase BDS 250 x 4.6 mm, 5µ. Mobile phase buffer: Acetonitrile in the ratio of 60:40and flow rate was maintained at 1ml/min, detection wave length was 267 nm, column temperature was set to 30°C and diluent was methanol:Water (50:50), Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 1.26 for repeatability and 0.93 for intermediate precision. LOD and LOQ are 0.080µg/ml and 0.243µg/ml respectively. By using above method assay of marketed formulation was carried out 100.24 % was present.

Keywords: HPLC, Crizotinib, Method development and ICH Guidelines.

INTRODUCTION

Crizotinib, is an anti-cancer drug acting as an ALK (anaplastic lymphoma kinase) and ROS1 (c-rosoncogene1) inhibitor, approved for treatment of some non-small cell lung carcinoma (NSCLC) in the US and some other countries, and undergoing clinical trials testing its safety and efficacy in anaplastic large cell lymphoma, neuroblastoma, and other advanced solid tumors in both adults and children. [1]

This compound belongs to the class of organic compounds known as pyrazolylpyridines. These are compounds containing a pyrazolylpyridine skeleton, which consists of a pyrazole linked (not fused) to a pyridine by a bond. [2]



Crizotinib structure

Synonym

US brand name: Xalkori; Code name: PF-02341066.[8]

IUPAC/Chemical name

(R)-3-(1-(2,6-dichloro-3-fluorophenyl)ethoxy)-5-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)pyridin-2-amine [2]

Chemical Formula

$C_{21}H_{22}C_{l2}FN_5O$ [2]

Crizotinib is an inhibitor of receptor tyrosine kinases including ALK, Hepatocyte Growth Factor Receptor (HGFR, c-Met), and Recepteur d'Origine Nantais (RON). Crizotinib has an aminopyridine structure, and functions as a protein kinase inhibitor by competitive binding within the ATP-binding pocket of target kinases. Crizotinib demonstrated concentration-dependent inhibition of ALK and c-Met phosphorylation in cell-based assays using tumor cell lines and demonstrated antitumor activity in mice bearing tumor xenografts that expressed EML4- or NPM-ALK fusion proteins or c-Met.[3]

MATERIALS AND METHODS

i. INSTRUMENT

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower

ii. Chemicals and Solvents

All the chemicals and solvents used were of analytical grade. Milli Q water was used throughout the experiment.

iii. Solutions

a) Preparation of Sample Solution[5, 9]

10 mg of crizotinib was accurately weighed and transferred into 20 ml of volumetric flask. The material is dissolved and diluted to mark with diluent viz. acetonitrile.

b) Assay Solutions[5, 9]

i. Standard Preparation-1

10.0 mg of crizotinib standard was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

ii. Standard Preparation-2

10.0 mg of crizotinib standard was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

iii. Assay Preparation-1

10.0 mg of crizotinib test sample was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

iv. Assay Preparation-2

10.0 mg of crizotinib test sample was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

c) Preparation of Buffer [5, 9]

Buffer

(0.1%OPA)

1mL of Ortho phosphoric acid solution in a 1000mL of Volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water.

d) Chromatographic Conditions

Column : Inertsil BDS-2, 150 x 4.6 mm, 5µm
Elution : Isocratic
Mobile Phase: Buffer: Acetonitrile (60:40 v/v)
Flow rate : 1.0 ml/min
Detector wavelength : 267 nm
Column oven temperature : 30 ºC
Injection volume : 20 µL
Diluent : Acetonitrile
Runtime : 30 min

RESULTS AND DISCUSSION

Method Development

Assay Procedure

Column is equilibrated for 30 min with mobile phase. 20 µL of diluent as blank was injected into the system and recorded the chromatogram for a run time of 30 min. 20 µl of standard preparation-1 was injected into the system and recorded the chromatogram for a run time of 30 min. 20 µl of standard preparation-2 was injected into the system and recorded the chromatogram for a run time of 30 min. Test is valid only when the match factor is in between 0.98 to 1.02. 20 µl of standard preparation-2 into the system was separately injected for four times and recorded each chromatogram for a run time of 30 min. Test is valid only when the five standard preparation-2 injections pass the system suitability. Average area of crizotinib peak was taken from five replicate injections and used in calculations. 20 µl of assay preparation-1 was injected into the system and recorded the chromatogram for a run time of 30 min. 20 µl of assay preparation-2 was injected into the system and recorded the chromatogram for a run time of 30 min. 20 µl of standard preparation-2 was injected into the system (online standard) and recorded the chromatogram for a run time of 30 min. % Assay (On dried basis) of the assay preparation-1 and assay preparation-2 was calculated separately.[9]

Optimized Chromatographic Conditions

Column : BDS (250*4.6 µm)
Mobile phase : OPA buffer: Acetonitrile (60:40)
Flow rate : 1.0 ml**/**min
Detector : PDA 267nm
Temperature : 30^0C
Injection Volume : 10µL

Fig 1: shows Crizotinib eluted with good peak shape and retention time and tailing was passed.

Method Validation [8, 10]

Linearity

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 37.5ppm to 225ppm of Crizotinib. Plot a graph to concentration versus peak area. Slope obtained was 21035 Y-Intercept was 892.5 and Correlation Co-efficient was found to be 0.999. Results were shown in Table 1 and Linearity plot was shown in Fig 2

Accuracy

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recover was calculated as 99.97% and %RSD was found to be 0.11 and Results were shown in Table 2 and chromatograms were shown in Fig 3-5.

Precision

Repeatability

Six working sample solutions of 100ppm are injected and the % Amount found was calculated and %RSD was found to be 1.26. Results were shown in Table 3 and chromatogram was shown in fig 6.

Intermediate precision

Six working sample solutions of 100ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.93. Results were shown in Table 4 and chromatogram was shown in fig 7.

Limit of Detection and Limit of Quantification

LOD and LOQ were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations,

$$LOD = 3.3 \times \sigma / s$$

and

$$LOQ = 10 \times \sigma / S.,$$

The results were presented in Table 5.

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

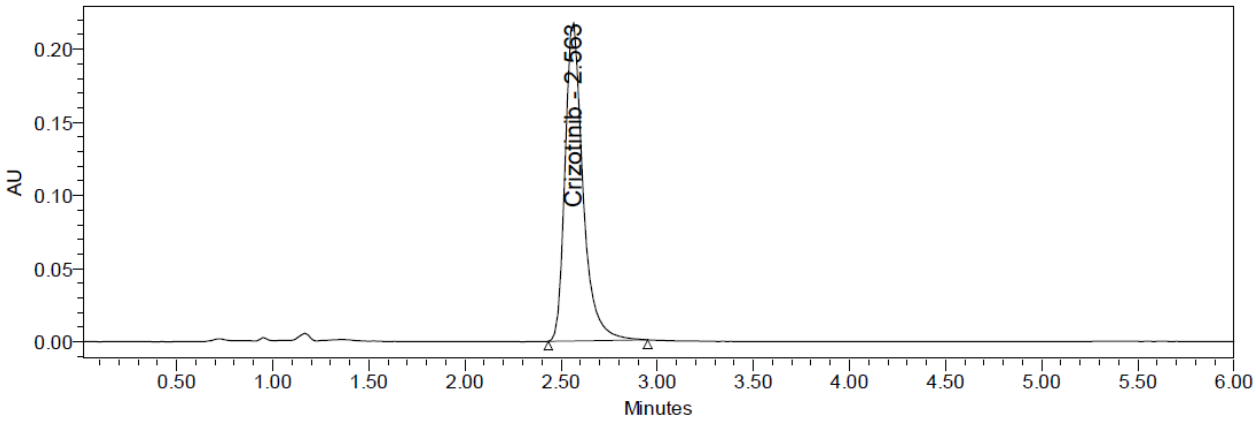


Fig 1: Optimized chromatogram

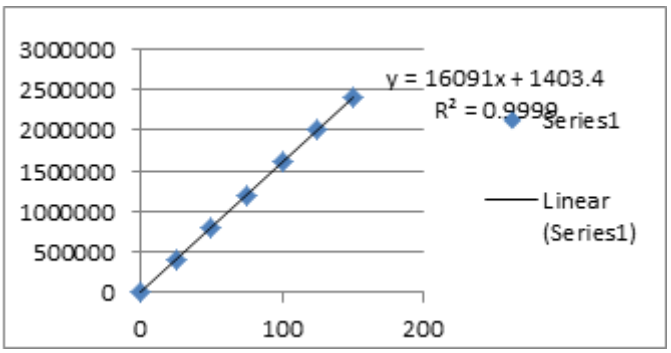


Fig. 2: Linearity Plot

Table 1: Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	25	400600
50	50	808783
75	75	1203917
100	100	1624787
125	125	2009776
150	150	2409818

Table 2: Accuracy data

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Crizotinib	50	50.6	101.2	2.1
	100	101.01	101.01	1.44
	150	150.37	100.25	1.36

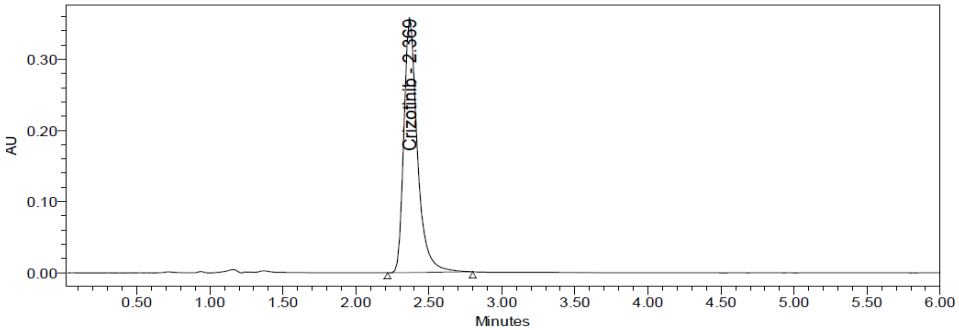


Fig. 3: Accuracy 50% Chromatogram

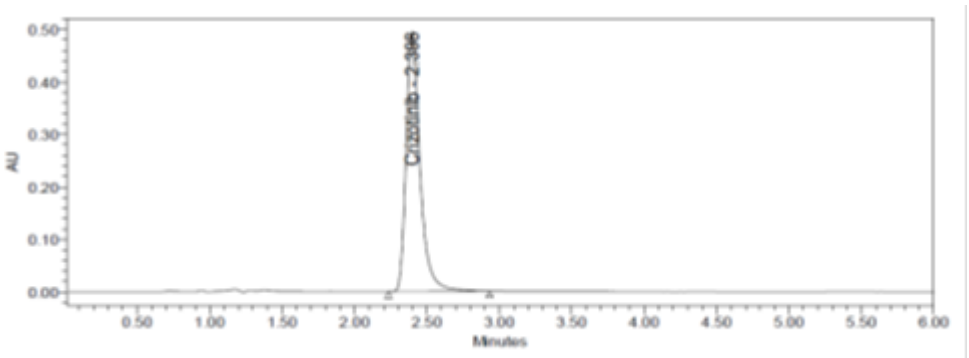


Fig. 4: Accuracy 100% Chromatogram

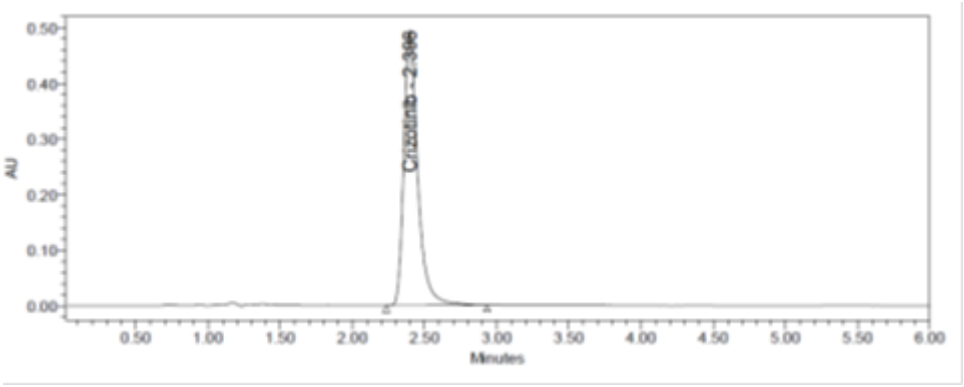


Fig. 5: Accuracy 150% Chromatogram

Table 3:
Repeatability data

S.No	Peak Area
1	1450617
2	1423806
3	1448914
4	1456113
5	1469303
6	1476272
AVG	1454171
STDEV	18349.4
%RSD	1.26

Table 4: Intermediate precision data

S.No	Peak Area
1	1439917
2	1470663
3	1472005
4	1477149
5	1473073
6	1462129
AVG	1465823
STDEV	13618.0
%RSD	0.93

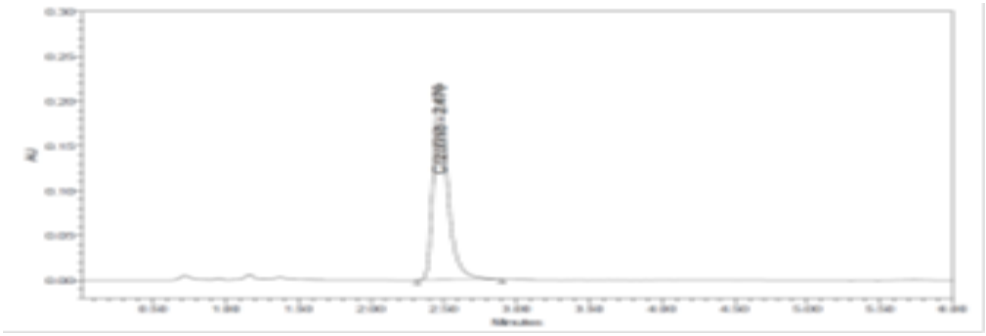


Fig. 6: Repeatability Chromatogram

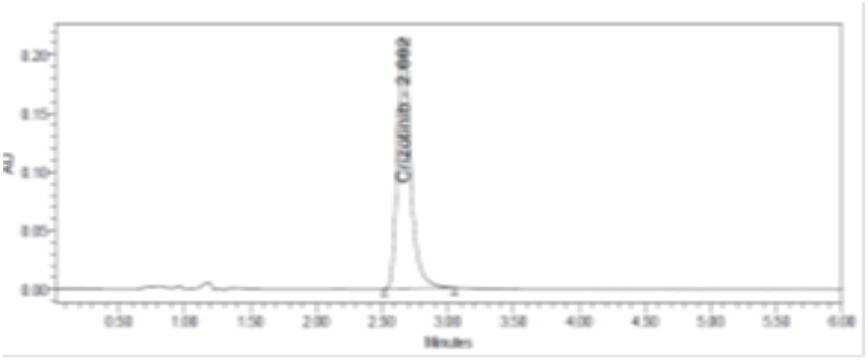


Fig. 7: Intermediate precision Chromatogram

Table: 5: Data table of LOD & LOQ for Bicalutamide and Atenolol

Drug	LOD (µg/ml)	LOQ (µg/ml
Crizotinib	0.080µg/ml	0.243µg/ml

CONCLUSION

Chromatographic conditions used are stationary phase BDS 250 x 4.6 mm, 5µ. Mobile phase buffer: Acetonitrile in the ratio of 60:40and flow rate was maintained at 1ml/min, detection wave length was 267 nm, column temperature was set to 30^{o}C and diluent was methanol:Water (50:50), Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 1.26 for repeatability and 0.93 for intermediate precision. LOD and LOQ are 0.080µg/ml and 0.243µg/ml respectively. By using above method assay of marketed formulation was carried out 100.24 % was present.

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