

## Analytical methods Development and Validation of Crizotinib by RP-HPLC Technique

Prapulla Putta\*

Department of Chemistry, Mother Teresa College of Pharmacy, N.F.C Nagar, Ghatkesar, Medchal, Telangana, India

### Article Info

**\*Corresponding author:****Prapulla Putta**

Associate Professor

Department of Chemistry

Mother Teresa College of Pharmacy, N.F.C

Nagar

Ghatkesar, Medchal, Telangana

India

Tel: +91-92914-42653

E-mail: prapullapharmacy@gmail.com

**Received:** December 17, 2018**Accepted:** January 18, 2019**Published:** January 28, 2019

**Citation:** Putta P. Analytical methods Development and Validation of Crizotinib by RP-HPLC Technique. *Int J Biochem Pharmacol.* 2019; 1(1): 1-4.  
doi: 10.18689/ijbp-1000101

**Copyright:** © 2019 The Author(s). This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Published by Madridge Publishers

### Abstract

A simple, précised, accurate method was developed for the estimation of Crizotinib by RP-HPLC technique. Chromatographic conditions used are stationary phase BDS 250 × 4.6 mm, 5 µ. Mobile phase buffer: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1 ml/min, detection wave length was 267 nm, column temperature was set to 30°C and diluents was methanol: water. System suitability parameters were studied by injecting the standard five times and results were well (50:50), conditions were finalized as optimized method under acceptance criteria. Linearity study was carried out between 25% to 150% 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 0.080 µg/ml. By using above method assay of marketed formulation was carried out 100.24% was present.

**Keywords:** Crizotinib; HPLC; ICH Guidelines; Method development.

### Introduction

Chromatography is a method of separating a mixture of components into individual components through equilibrium distribution between two phases [1]. The technique of chromatography is based on the differences in the rate at which components of a mixture move through a porous medium (stationary phase) under the influence of some solvent or gas (mobile phase) [2]. Crizotinib: Crizotinib, is an anti-cancer drug acting as an ALK (anaplastic lymphoma kinase) and ROS1 (c-rosoncogene1) inhibitor [3]. The aim and objective of the work is to develop and validate a simple and economical RP-HPLC method as per ICH guidelines for the estimation of Crizotinib in drug and pharmaceutical dosage forms, and to study the linearity of the method (Figure 1).

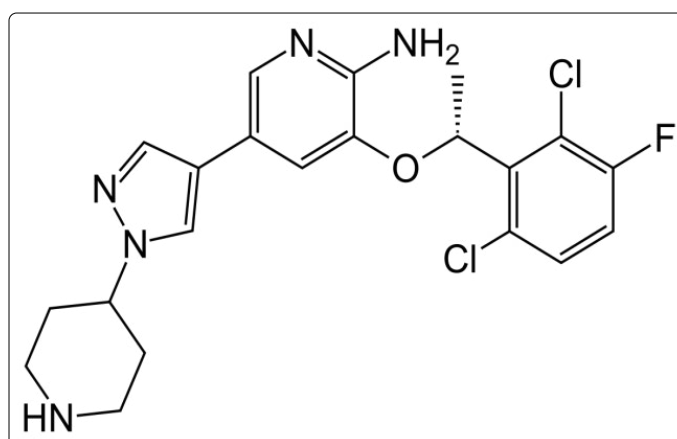


Figure 1. Structure of Crizotinib.

## Materials and Methods

Crizotinib was obtained as a gift sample from Wintac Ltd., (Bangalore). Acetonitrile served as solvent mixture was also obtained from CDH, New Delhi. All other chemicals/reagents were of analytical grade and were used without further purification.

### Preparation of buffer

(0.1% OPA): 1 ml of Ortho phosphoric acid solution in a 1000 ml of volumetric flask is taken and add about 100 ml of milli-Q water and final volume make up to 1000 ml with milli-Q water [4].

### Optimized chromatographic conditions

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

### Method of validation

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, robustness, ruggedness, sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines [5].

### Method of linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n=3) at a concentration range of 2-20 μg/ml. The absorbance obtained at respective concentration was recorded, and the graph is plotted as concentration (μg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software [6].

### Method of accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 80%, 100% and 120%. The final concentration of Crizotinib was determined at each levels of the amount; three determinations were performed.

The percentage recovery was calculated as mean ± standard deviation [7].

### Method of precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day precision study, three different solutions of same concentration were prepared and analysed in the same day (morning, noon and evening), whereas in the inter-day precision study, the solutions of same concentration were prepared and analysed, for three consecutive days, and the absorbance were recorded. All study was performed in triplicates. The result was indicated by calculating percentage RSD [8].

### Method of robustness

The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [9].

### Method of ruggedness

The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days [10].

### 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 (Figure 2). 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 [11] (Figure 3).

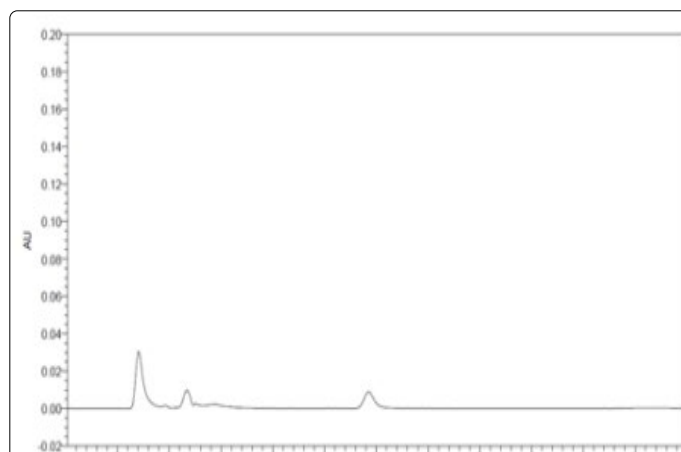


Figure 2. Blank Chromatogram.

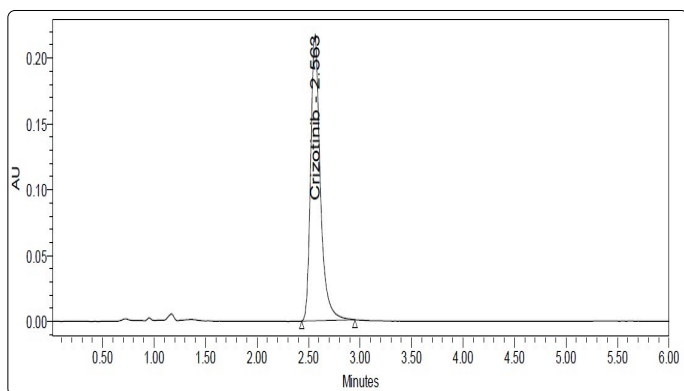


Figure 3. System Suitability Chromatogram.

## Results and Discussion

Results were shown in the below given tables and figures. Table 1 shows system suitability data.

Table 1. System suitability-System suitability data

| Sl. No             | Peak Name  | RT    | Area    | USP Plate Count | USP Tailing |
|--------------------|------------|-------|---------|-----------------|-------------|
| 1                  | Crizotinib | 2.556 | 1458811 | 3949            | 1.37        |
| 2                  | Crizotinib | 2.563 | 1412479 | 3927            | 1.34        |
| 3                  | Crizotinib | 2.565 | 1450647 | 3811            | 1.33        |
| 4                  | Crizotinib | 2.574 | 1465621 | 3719            | 1.37        |
| 5                  | Crizotinib | 2.577 | 1463605 | 3784            | 1.35        |
| 6                  | Crizotinib | 2.617 | 1444216 | 3885            | 1.33        |
| Mean               |            |       | 1449230 |                 |             |
| Standard deviation |            |       | 19722.4 |                 |             |
| % RSD              |            |       | 1.4     |                 |             |

### Method of precision

Table 2 shows the repeatability data.

Table 2. Repeatability data

| Sl. No    | Peak area |
|-----------|-----------|
| 1         | 1450617   |
| 2         | 1423806   |
| 3         | 1448914   |
| 4         | 1456113   |
| 5         | 1469303   |
| 6         | 1476272   |
| Avg.      | 1454171   |
| Std. devi | 18349.4   |
| % RSD     | 1.26      |

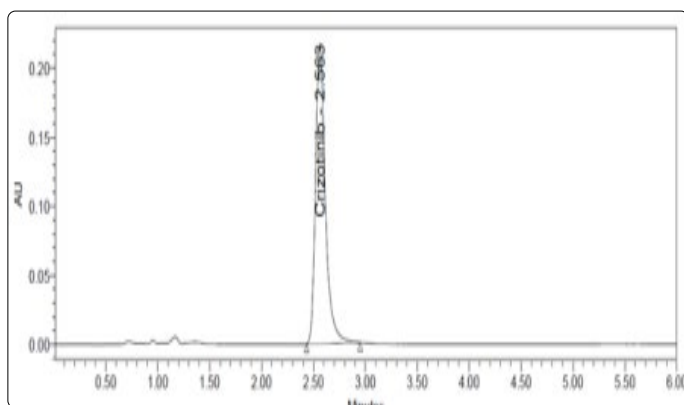


Figure 4. Optimized Chromatogram.

### Observation

Crizotinib eluted with good peak shape and retention time and tailing was passed (Figure 4).

### Intermediate precision (Table 3)

Six working sample solutions of 100 ppm 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 and chromatogram was shown in figure 3.

Table 3. Intermediate precision data

| Sl. No             | Peak Area |
|--------------------|-----------|
| 1                  | 1439917   |
| 2                  | 1470663   |
| 3                  | 1472005   |
| 4                  | 1477149   |
| 5                  | 1473073   |
| 6                  | 1462129   |
| Average            | 1465823   |
| Standard deviation | 13618.0   |
| % RSD              | 0.93      |

### Method of linearity

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 37.5 ppm to 225 ppm 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 and Linearity plot (Tables 4 and 5).

Table 4. Linearity Concentration and Response

| Sl. No | Linearity Level (%) | Concentration (ppm) | Area    |
|--------|---------------------|---------------------|---------|
| 1      | 0                   | 0                   | 0       |
| 2      | 25                  | 25                  | 400600  |
| 3      | 50                  | 50                  | 808783  |
| 4      | 75                  | 75                  | 1203917 |
| 5      | 100                 | 100                 | 1624787 |
| 6      | 125                 | 125                 | 2009776 |
| 7      | 150                 | 150                 | 2409818 |

Table 5. Summary of results.

| Parameters                                | Crizotinib      |
|---|-----------------|
| Calibration range (mcg/ml)                | 25-150 ppm      |
| Optimized wave length                     | 267 nm          |
| Retention time                            | 2.563 min       |
| Regression equation (Y)                   | y=16091x+1403.4 |
| Correlation coefficient (r <sup>2</sup> ) | 0.999           |
| Precision (% RSD*)                        | 1.26            |
| % Recovery                                | 100.24          |
| Limit of Detection (µg/ml)                | 0.080           |
| Limit of Quantization (µg/ml)             | 0.243           |

## Conclusion

Chromatographic conditions used are stationary phase BDS 250 × 4.6 mm, 5 µ. Mobile phase buffer: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1 ml/min, detection wave length was 267 nm, column temperature was set to 30°C and diluents 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% to 150% levels, r<sup>2</sup> 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 0.080 µg/ml and 0.243 µg/ml respectively. By using above method assay of marketed formulation was carried out 100.24% was present.

## References

1. Sethi PD. Quantitative analysis of Drugs & Pharmaceuticals. 3<sup>rd</sup> edition, CBS publishers and distributors, New Delhi. 2001: 1-120.
2. PubChem. Compound Summary for CID 11626560: Crizotinib. Open Chemistry Database.
3. Ramachandra B, Naidu NVS. Validation of RP -HPLC Method for Estimation of Dasatinib In Bulk and Its Pharmaceutical Dosage Forms. *Int J Pharm Bio Sci.* 2014; 4(1): 61-68.
4. Konam K, Jadhav D. Development and validation of a RP- HPLC method for determination of dronedarone in bulk and pharmaceutical formulation *Int J Pharm Bio Sci.* 2014; 4(1): 179-185.
5. International Conference on Harmonization (ICH). Q2A Validation of Analytical Procedures: Methodology. Geneva. 1994.
6. Skoog DA, Holler FJ, Nieman TA. Principles of Instrumental Analysis. 7<sup>th</sup> edition, Cengage Learning. 2017: 973-995.
7. Rajyalakshmi Ch, Benjamin T, Rambabu C. Forced degradation study on dronedarone and application of validated stability - indicating HPLC -UV method in stability testing of dronedarone tablets. *Der Pharma Chemica.* 2013; 5(1): 189-195.
8. Selvakumar S, Ravichandran S, Matsyagiri L. Development and Validation of analytical method for Simultaneous estimation of Ornidazole and Cefixime trihydrate tablet dosage forms by UV spectroscopy. *Asian J Pharm Ana.* 2016; 6(4): 246-252.
9. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. 5<sup>th</sup> Revised and Enlarged edition, Himalaya Publication House, Mumbai. 2002; 2: 630.
10. Matsyagiri L, Jagadeesh P, Mounika B, et al. Effect of solvents on Spectrophotometric Estimation of Tinidazole in bulk and dosage forms. *World Journal of Pharmaceutical Research.* 2018; 7(9): 1742-1754.
11. Jadhav PB, Shejwal V. Development and Validation of an RP-HPLC Method for Crizotinib. *IJPPR Human.* 2017; 9(2): 100-106.