



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article

May 2017 Vol.:9, Issue:2


© All rights are reserved by P. B. Jadhav et al.

Development and Validation of an RP-HPLC Method for Crizotinib



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203



P. B. Jadhav*, Vivek Shejwal¹

*Dr. Babasaheb Ambedkar Marathwada University,
Aurangabad, Maharashtra, India*

Submission: 5 May 2017
Accepted: 10 May 2017
Published: 25 May 2017

Keywords: Crizotinib, RP-HPLC, Method development, Validation.

ABSTRACT

An accurate, Precise, Simple and Economical High-Performance Liquid Chromatographic method for the estimation of Crizotinib in bulk form has been developed. The method so developed is Reverse-Phase High-Performance Liquid Chromatographic method using Prime's C18 column (Length: 250mm, Diameter: 4.6mm, Particle size:5 μ) with a simple methanol and Sodium Phosphate Buffer 10 mmph 6.5 mixed in a proportion of 85:15 v/v as the mobile phase. The retention time for Crizotinib was found to be 7.34 min. The linearity for the method was observed in a concentration range of 10-50 μ g/mL with the correlation coefficient of 0.998. The method so developed was validated in compliance with the regulatory guidelines by using well developed Analytical method validation tool which comprises with the analytical method validation parameters like Linearity, Accuracy, Method precision, Specificity, System suitability, Robustness, and Ruggedness. The results obtained were well within the acceptance criteria.



HUMAN JOURNALS

www.ijppr.humanjournals.com

INTRODUCTION

Crizotinib is tyrosine kinase inhibitor, as there are few other analytical methods available for the estimation of this drug in the bulk and pharmaceutical dosage forms. The chemical structure of crizotinib is given in Fig 1. Chemically it is (R)-3-[1-(2,6-Dichloro-3-fluorophenyl)ethoxy]-5-[1-(piperidin-4-yl)-1H-pyrazol-4-yl]pyridin-2-amine. With empirical formula $C_{21}H_{22}Cl_2FN_5O$. In the present work simple, accurate and precise RP-HPLC method has been developed and validated.

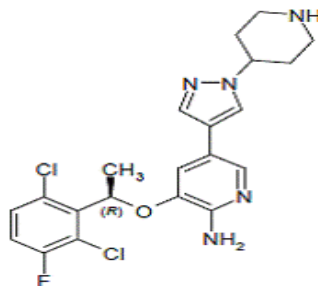


Fig 1: Chemical Structure of Crizotinib

MATERIALS AND METHOD

Instrumentation



Chromatographic separation was achieved using a C-18 column (250mm x 4.6mm i.d., 5 μ m particle size) of Young Lin (S.K) Gradient system that is equipped with UV Detector

Materials Required

Crizotinib pure standard was purchased from Swapnaroop drug agency (India). Methanol and Water of HPLC grade were purchased from Merck (India) and Qualigens (India) respectively. Crizotinib tablets available under the brand name xalkori (Pfizer Ltd) were purchased and used. Optimized Conditions the mobile phase with methanol and Sodium Phosphate Buffer in the ratio of 85:15 %v/v was employed in isocratic mode at a flow rate of 0.7 ml/min. The run time was 10 mins and 20 μ L of the sample was injected for every run into the column. The wavelength of the UV detector was set at 266nm.

Chromatographic conditions

A mixture of phosphate buffer and methanol in the ratio of 85:15 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Crizotinib. The solvent mixture

was filtered through 0.45 μ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.7mL/min. Injection volume was 20 μ L and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 266 nm. The run time was set at 10min.

Preparation of Standard Stock Solution

Accurately about 10mg of Crizotinib was weighed and transferred to a 10mL volumetric flask. 5mL of methanol was added to the flask and sonicated to dissolve it. The volume was then made up to the mark with methanol to get a standard solution of Crizotinib at a concentration of 1000 μ g/mL

Preparation of sample Solutions

Working solutions for HPLC injections were prepared on daily basis. Aliquots of the standard stock solution were taken and diluted with the mobile phase to get solutions in a concentration range of 10-50 μ g/mL.



Linearity

Several aliquots of standard solution of Crizotinib was taken in different 10ml volumetric flasks and diluted up to the mark with diluents such that the final concentrations of Crizotinib were in the range of 10 to 50 μ g/mL. Evaluation of the drug was performed with UV detector at 266 nm, peak area was recorded for all the peaks. The correlation coefficient value of Crizotinib was 0.998. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The data is tabulated in table 1.

System suitability

System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (80,100 and 120%) and the percentage recoveries were calculated. The data is tabulated in table 2.

Precision

The precision of the method was determined by Intra and inter-day precision studies. This was evaluated by injecting three independent sample preparations of Crizotinib from a single formulation at three different concentration levels on the same day (Intra-day) and on three different days (Inter-day). The %RSD was then calculated. The data is represented in table 3.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values.

Robustness

Robustness was established by introducing small changes in the HPLC optimized conditions which include mobile phase ratio (± 1), flow rate ratio (± 0.1) and wavelength (± 1). This was studied using two replicates at a concentration level of 40 $\mu\text{g/mL}$ of Crizotinib.

Table 1: Data for Linearity

Conc. ($\mu\text{g/mL}$)	Avg. peak Area
10	518.20
20	1083.73
30	1512.37
40	2064.20
50	2520.65

Table 2: Recovery Studies for Crizotinib

% Spike Level	Amount Added ($\mu\text{g/mL}$)	Peak Area	Amount Found ($\mu\text{g/mL}$)	% Recovery	Mean % Recovery
80	8	939.44	17.95	99.48	98.58
80	8	932.27	17.81	97.68	
100	10	1036.8	19.91	99.11	98.94
100	10	1035.11	19.87	98.77	
120	12	1124.73	21.67	97.29	98.06
120	12	1134.37	21.86	98.83	

Table 3: Data for Precision

Sr. No.	Conc.(µg/ml)	Inter-day		Intra-day	
		Mean Area*±SD	%RSD	Mean Area*±SD	%RSD
1	10	545.18±3.60	0.66	544.03±6.59	1.21
2	30	1567.62±3.09	0.20	1545.30±14.27	0.92
3	50	2531.00±10.34	0.41	2556.72±5.10	0.20

*Mean area of two injections.

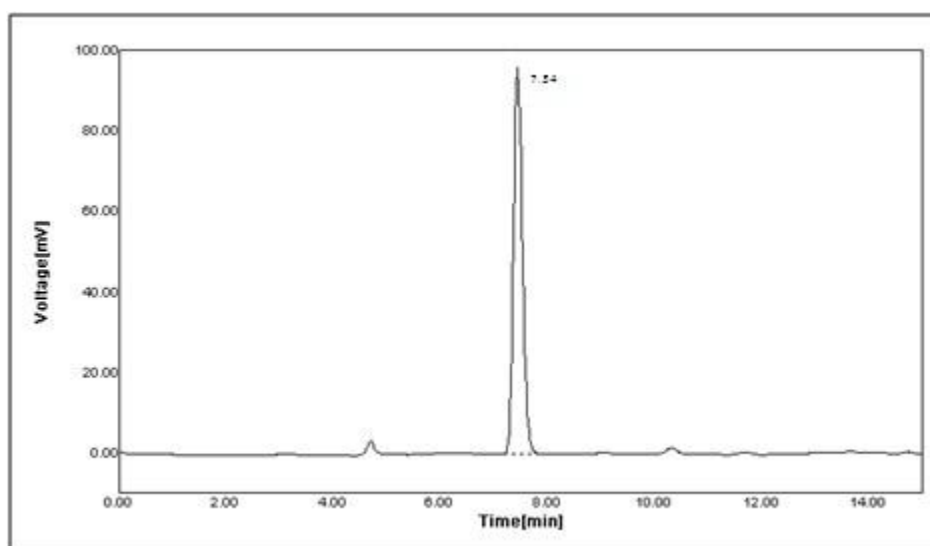


Fig 2: Chromatogram of Crizotinib

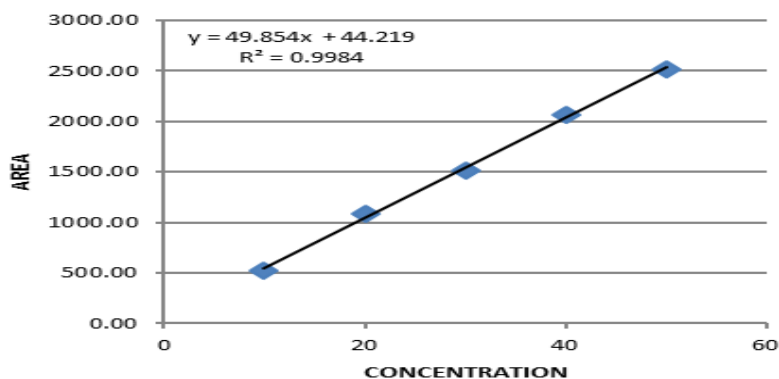


Fig 3: Calibration Curve of Crizotinib

RESULTS AND DISCUSSION

The proposed method was found to be simple. Linearity was observed in the concentration range of 10-50 $\mu\text{g/mL}$ with the regression equation $y=49.85x+ 44.21$ and the correlation coefficient of 0.998. System suitability parameters indicate high column efficiency with a large number of theoretical plates (>2000). The tailing factor was found to be 1.21 which does not exceed the critical value (2). The average retention time was found to be 7.34. No interference was seen from any of the components of the pharmaceutical dosage form indicating the specificity of the method. The recovery studies were performed and the % RSD was found to be in the range 0.24-1.29. The % RSD was found to be 0.199-1.21 for intraday and 0.197-0.66 for inter-day precision studies. Thus the method was found to be accurate and precise as the % RSD was not more than 2%. The limit of detection and limit of quantification for Crizotinib were found to be 0.297 $\mu\text{g/ml}$ and 0.901 $\mu\text{g/ml}$ respectively. The RSD for the % assay of the sample was calculated for each parameter in robustness and was found to be less than 2% confirming the robustness of the method.



CONCLUSION

A validated RP-HPLC method was developed for the determination of Crizotinib in tablet dosage form and bulk forms. As the proposed method is simple, rapid, accurate, precise and specific it can be employed for the routine analysis of Crizotinib in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

I express my sincere thanks to reliable institute and swapnaroop drug agency, Aurangabad for providing me all the facilities and also to my friend Vivek for helping me at every time. Last but not most important, thanks to my Mom and Dad.

REFERENCES

1. K. Kalekar, B. A. Rao, Y. Allamneni, P. D. Chary, S. S. Kumar, N. Allamneni, Development and Validation of RP-HPLC Method for Estimation of Dasatinib in bulk and its Pharmaceutical formulation, American Journal of Pharmtech Research, 2012, 863-872, 2249-3387.
2. K. Kuna, J. K. Kuna, RP-HPLC Method Development And Validation Of ImatinibMesylate in Tablet Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences, 2011, 162-165, 0975-1491.
3. R. Gennaro, B. M. Karen, T. Medwick, Remington: The Science and Practice of Pharmacy, 19th ed.; Vol-I, The

- Mack Publishing Company, Pennsylvania, 1995, pp 437-490.
4. A. Weston, P.R. Brown, High Performance Liquid Chromatography, Separations in High Performance Liquid Chromatography, Instrumentation for HPLC. In: HPLC and CE – Principles and practice. Academic Press, USA, 1997, pp 1-11, 24-32.
 5. Analytical chemistry. http://lingualeo.com/es_LA/jungle/analytical-chemistry-163830#/page/1 (accessed on Jan 07, 2016).
 6. K. Sharma, Instrumental Method of Chemical Analysis, 12th ed.; MerrutGoel Publishing House, 1992, pp 56-126.
 7. Ramachandra and N.V.S. Naidu, Validation of RP-HPLC Method for Estimation of Dasatinib In Bulk and Its Pharmaceutical Dosage Forms, International journal of pharmacy and biological sciences, 2014, 61-68, 2230-7605.
 8. Crizotinib. <https://pubchem.ncbi.nlm.nih.gov/compound> (accessed Oct 21, 2015).
 9. A. Skoog, F. J. Holler, S. R. Crouch, Principle of Instrumental Analysis, 6th ed.; Thomson Publications, India, 2007, pp 1-6, 145-180.
 10. Pirro, S. Francia, F. Martino, C. Fava, S. Ulisciani, G. Cambrin, S. Racca, G. Saglio, and F. Carlo, A New HPLC–UV Validated Method for Therapeutic Drug Monitoring of Tyrosine Kinase Inhibitors in Leukemic Patients, Journal of Chromatographic Science, Vol. 49, 2011, 753-757.
 11. R. Chatwal, S. K. Anand, Instrumental Methods of Chemical Analysis. 5th ed.; Himalaya Publishing House, 2002, pp 107-110.
 12. G.D. Christian, Analytical Chemistry, 7th ed.; John Wiley and Sons, 2003, pp 5-42, 131-132.
 13. H. Willard, L. L. Merritt, J. A. Dean, F. A. Settle, Instrumental Methods of Analysis, 7th ed.; CBS Publisher and Distributors, New Delhi, 2002, pp 617.
 14. ICH Guidelines on Validation of Analytical procedure: Text and Methodology Q 2 (R1), (2011). [RJC-761/2011].
 15. Indian pharmacopoeia, (1996) Vol-2. Controller of Publications, Ministry of health and family welfare (131-132), Delhi, Government of India.
 16. M. Harika, G. S. Kumar, Development And Validation of RP-HPLC Method for Estimation of Nilotinib In Bulk and Its Pharmaceutical Formulation, international research journal of pharmacy, 2012, 2230 – 8407.
 17. M. Dziadosz, R. Lessiga, H. Bartels, HPLC–DAD protein kinase inhibitor analysis in human serum, J. Chromatography B. Analyt Technology Biomed Life Science, 2012, 77–81, 893–894.
 18. M. W. Dong, Modern HPLC for practicing scientist, 1st ed.; A John Wiley & Sons Interscience Publication, 2007, pp 194-217.
 19. Online medical resource. <http://www.rxlist.com/bosulif-drug.htm> (accessed Nov 06, 2015).
 20. P. C. Kamboj, Introduction in Pharmaceutical Analysis. Volume I, 2nd ed.; Vallabh Publication, 2005, Delhi, pp 1-3.
 21. P. D. Sethi, High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations, 1st ed.; CBS Publishers and Distributors, New Delhi, 2007, pp 1-11, 116-120.
 22. P. S. Kalsi, Spectroscopy of Organic Compounds. 6th ed.; New Age International Publishers, 2007, pp 7-10.
 23. P. Sandhya, P. Vishnu Priya, Shyamala, N. Anjali Devi, JVC. Sharma, Method Development And Validation of Imatinib Mesylate In Pharmaceutical Dosage Form By RP-HPLC, World journal of pharmacy and pharmaceutical science vol.3, 2013, 682-688, 2278-4357.
 24. R. Malviya, V. Bansal, O. P. Pal and P. K. Sharma, High performance liquid chromatography: a short review, J. global pharma technology, 2009, 22-26, 0975– 8542.
 25. R. Snyder, Practical HPLC method development. 2nd ed.; Wiley Interscience Publication: 1997. pp.120, 234-240, 266-278, 688.
 26. R.P.W Scott, Technique and Practice of chromatography, Marcel Dekker, Vol. 70.; New York, 1995, pp 1-12.
 27. R.S. Phani, K.R.S. Prasad and U Reddy, Scientific approach for RP-HPLC method development: complete review, vol 2, J. Caribbean Journal of Science and Technology, 2016, 896-903, 2249-5347.
 28. S. M. Khopkar, Basic concepts of analytical chemistry, 2nd ed.; New age International Ltd. Publishers, New Delhi, 1998, pp 178-179.
 29. United State Pharmacopoeia, USP 29/NF 24, US Pharmacopoeial Convention, Inc, Rockville MD, 2006, 555-556, 1777, 1861, 3050-3052.
 30. V.K. Chakravarthy and D.G. Sankar, Development and validation of RP-HPLC method for estimation of Erlotinib in bulk and its pharmaceutical formulation,
 31. J. Rasayan Vol.4, 2011, 393-399, 0974-1496.