Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublisher.com/sajp/

OPEN ACCESS

Pharmacy

Analytical Method Development and Validation of Vandetanib by Using RP-HPLC of Bulk Drug

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DOI: 10.21276/sajp.2019.8.8.8 | **Received:** 13.08.2019 | **Accepted:** 20.08.2019 | **Published:** 21.08.2019

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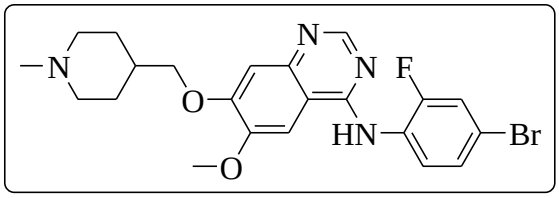
Abstract **Original Research Article**

RP-HPLC is fast, simple, sensitive, precise, and reproducible (liquid chromatography) method was developed and validated for the analysis of vandetanib bulk dosage form. Using C-18 HPLC column separation was carried out. Which was maintained at ambient temperature. During separation mobile phase consist of methanol (100 v/v) was delivered at a rate of 1mL/min. Analysis was carried out by using UV detector at the wavelength 328 nm. In RP-HPLC method was validated by using various parameters like, precision, limit of quantitation (LOQ), linearity and robustness. The RP-HPLC method was found to be linear over the concentration ranges from 50-100 µg/mL (r^2 =0.9996). Retention time for bulk vandetanib was found to be 5.496±25 min. LOQ of method was 6.8339 µg/mL and LOD 2.7036µg/mL. Thus, the RP-HPLC developed method was found to be robust and rugged which can be applied for the regular analysis of vandetanib in the bulk as well as pharmaceutical dosage form.

Keywords: C18, RP-HPLC, Methanol, Vandetanib.

INTRODUCTION

Vandetanib(N(4-bromo-2-fluorophenyl))6-methoxy-7[(1-methyl-4-piperidinyl)methoxy]4-quinazolinamine) is a recently identified small molecule inhibitor, which shows antitumor efficacy by inhibiting tumor cell proliferation and survival via epidermal growth factor receptor (EGFR) and RET inhibition, as well as inhibiting tumor angiogenesis via vascular EGFR-2 (VEGFR-2) inhibition. Its preclinical and clinical activity against several tumor types including advanced and metastatic papillary thyroid cancer, non-small cell lung cancer (NSCLC), and advanced colorectal cancer (CRC) either in monotherapy or in combination with other anticancer agent as first or secondline therapy has been demonstrated.



In literature survey reveals that a few spectrophotometric, RP-HPLCmethods are reported for the estimation of Vandetanib in combination with other drugs.Main purpose of this work to develop a RP-HPLC method for the determination of vandetanib in bulk form for the provide more scope in further research study on the drug and pharma industry [1-4].

MATERIALS AND METHOD

Chemicals

Vandetanib was received as a gift sample from Mylan Laboratories Limited, Hyderabad, India. HPLC grade methanol and double distilled water as solvent was used for the other purpose.

Instrumentation

The chromatographic technique was performed on a Shimadzu LC-2010C_{HT} Liquid chromatography with UV-visible detector and LC-Solution software, reversed phase C18 column (Inerstil ODS-3V 5 um 250×4.6 mm), Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study. Double beam UV-visible spectrophotometer (UV-probe 2.32 software) [5].

Determination of Working Wavelength (λmax)

10 mg of vandetanib was weighed and transferred in to 100 mL volumetric flask and dissolved in methanol and then make a dilution of that stock solution. Prepare 10 µg/mL solution by diluting 1 mL to

10 mL with methanol. Wavelength of maximum absorption for 10 µg/mL solution of the bulk drug in methanol was scanned using UV-Visible spectrophotometer within the range of 200-400 nm wavelength with methanol as reference. The absorption curve shows at 328 nm for vandetanib bulk [6-20].

Chromatographic Condition

Mobile phase for this developed method methanol 100% and it was filtered through a 0.45µmmembrane filter degassed with a helium spurge for 30min and pumped from the respective solvent reservoir to the column inerstil C18 column (250×4.6 mm) at flow rate 1.0 mL/min.This HPLC developed method run time was set at 10 min and column temperature was maintained at RT. Then prior to inject the bulk drug solution in to the column, column was equilibrated for at least 30 min with the same mobile phase flowing through the system.The eluent was monitored at 328 nm. Using "LC-Solution" software data was stored and analysed [2].

Selection of Mobile Phase

At the beginning solution of bulk drug vandetanib was injected into the HPLC system and run in various solvent system. Various mobile phase methanol, water, acetonitrile, and phosphate buffer in different ratio were tried and finally methanol (100%) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for bulk vandetanib [20].

Evaluation of Analytical Methods

Linearity

For the linearity study were prepared suitable dilution (ranging from 50-100 µg /mL) of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for vandetanib and higher concentration range was used to improve signal to noise ratio. Linearity was determined by analysing five working standard solutions over the concentration range of 50-100 µg /mL for vandetanib [20].

Limit of Detection (LOD)

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula:

$$LOD = 3.3\sigma/s$$

Where,

σ = standard deviation of the response

S = slope of the calibration curve.

Limit of Quantification (LOQ)

The limit quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula:

$$LOQ = 10\sigma/s$$

Where,

σ = standard deviation of the response

S = slope of calibration curve

Accuracy

Accuracy of this method was carried out using one set of different standard addition methods at different concentration levels 80%, 100% and 120%, and then comparing the difference between the spiked value (theoretical value) and actual found value [10, 11].

Precision

Five sets of aliquots with same concentration (90 µg /mL) were prepared and these solutions were analysed to record any intra and inter day variations in the results. The results obtained for Intra and inter day variations.

Robustness

Robustness of the proposed method for vandetanib was carried out by the slight variation in flow rate, temperature and mobile phase ratio. The percentage recovery and RSD were noted for vandetanib.

RESULT AND DISCUSSION

Checking Resolution of Drug and Materials

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of vandetanib was injected to get the chromatogram. The retention time for vandetanib was found to be 5.496 min. It is shown in the Table-1.

Table-1: Resolution of bulk drug

Drug	Ret. Time	Area	Height	Theoretical plate	Tailing factor
Vandetanib	5.496	35957814	340511	3206.216	1.387

Linearity

The data of the peak area vs drug concentration were evaluated by linear regression analysis as shown in the Table-2 and calibration curve obtained after plotting drug concentration vs area shown in the Fig-1. Linear regression analysis demonstrated that chromatograph response for the drug was highly linear (r^2=0.9996) in the studied concentration range of 50-100 µg/mL. A typical

chromatogram of vandetanib (50 µg/mL) shown in Fig-1.

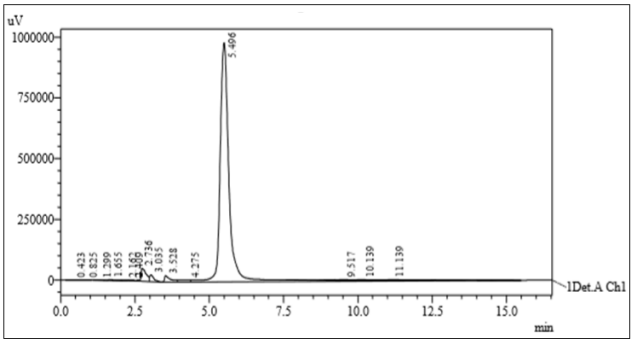


Fig-1: A typical chromatogram for Vandetanib (50µg/mL)

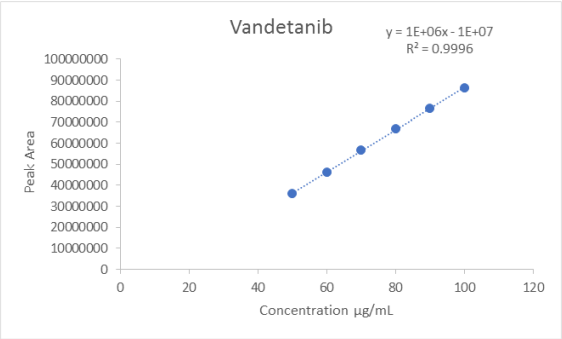


Fig-2: Calibration curve of Vandetanib

Table-2: Calibration of Vandetanib

Sr No	Concentration (µg /mL)	Peak area
1	50	35957814
2	60	46217278
3	70	56914020
4	80	66967139
5	90	76629861
6	100	86282828

Precision

The result depicted in the Table 3a & 3b indicated that the given method has sufficient precision as indicated by the corresponding values of %RSD ranging 0.13 for inter day studies respectively. The values of %RSD for both the studies are well below 1.0% constructing adequate precision.

Table-3a: Intra-day Precision for Vandetanib

Concentration (µg /mL)	Peak area	Mean (n=5)	S.D.	%RSD
90	76629865			
90	77620844			
90	76528703	76618861	12622.96	0.13
90	77429855			
90	76069865			

Table-3b: Inter-day Precision for vandetanib

Concentration (µg /mL)	Peak area	Mean (n=5)	S.D.	%RSD
90	75629865			
90	76620844			
90	75528703	75100844	38217.53	0.16
90	75429855			
90	77069865			

Limit of Detection and Quantification

Standard error and slope of linear data is used to predict LOD and LOQ of rivastigmine and precision was established at the predict concentration. The result was shown in the Table-4.

Table-4: Limit of detection and Limit of quantification

Limit of Detection	Limit of Quantification
2.7036µg/mL	6.8339µg/mL

CONCLUSION

From the results and discussion, RP-HPLC methods were developed and validated as per ICH guidelines Q2 (R1). In this paper described for the determination of vandetanib in bulk is simple, sensitive and reproducible. The proposed methods can be successfully applied for vandetanib without any interference in quality control.

ACKNOWLEDGEMENTS

The authors are grateful to, the Principal and the Management of Kasturi Shikshan Sanstha College of Pharmacy Shikrapur Pune. The authors are thankful to mylan laboratories pvt. ltd., Hyderabad (India) for providing gift sample.

REFERENCE

1. Darwish HW, Bakheit AH. A new spectrofluorimetric assay method for vandetanib in tablets, plasma and urine. Tropical Journal of Pharmaceutical Research. 2016;15(10):2219-25.
2. Andriamanana I, Gana I, Duretz B, Hulin A. Simultaneous analysis of anticancer agents bortezomib, imatinib, nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS. Journal of Chromatography B. 2013 May 1;926:83-91.
3. Amer SM, Kadi AA, Darwish HW, Attwa MW. Liquid chromatography tandem mass spectrometry method for the quantification of vandetanib in human plasma and rat liver microsomes matrices: metabolic stability investigation. Chemistry Central Journal. 2017 Dec;11(1):45.
4. Lin H, Cui D, Cao Z, Bu Q, Xu Y, Zhao Y. Validation of a high-performance liquid chromatographic ultraviolet detection method for the quantification of vandetanib in rat plasma and its application to pharmacokinetic studies. Journal of cancer research and therapeutics. 2014 Jan 1;10(1):84.
5. Dudhe PB, Choudhary ED. Development and Validation of First Order Derivative Method for Tenofovir alafenamide in Bulk using UV Visible Spectroscopy. International Journal Chemical Technology Research, 2018; 11(8), 267-273.
6. Dudhe PB, Sonawane AM. Spectrophotometric Determination of Cycloserin in Bulk and Capsule Dosage form by Area Under Curve and First Order Derivative Methods. International Journal of pharmtech Research. 2016;9(8):131-9.
7. Dudhe PB, Kamble MC, Van S, Rajpurohit VJ, Komerwar A, Gondane SJ. Development and Validation of a Spectrophotometric Method for Glibenclamide in Bulk and Tablet Dosage Forms. International Journal of Pharm Tech Research. 2016; 9(2):19-23.
8. Dudhe PB, Shelke P, Chavare P. Determination of Apixaban from Bulk and Tablet Dosage Form by Area Under Curve and First Order Derivative Spectrophotometric Methods. International Journal of ChemTech Research. 2017;10(5):703-11.
9. Dudhe PB, Shinde AP, Salgar K. Development and validation of analytical methods for Simultaneous estimation of domperidone and esomeprazole Magnesium in bulk and in pharmaceutical formulations Using UV-Visible spectroscopy. International Journal of PharmTech Research. 2014;6(5):1501-8.
10. PB D, Kamble MC, Komerwar A, Sonawane AM, Van S. Development and Validation of First Order Derivative Method for Metronidazole in Bulk and Tablet Using UV Visible Spectroscopy. 2016.
11. Devrukhakar PS, Borkar R, Shastri N, Surendranath KV. A validated stability-indicating RP-HPLC method for the simultaneous determination of tenofovir, emtricitabine, and a efavirenz and statistical approach to determine the effect of variables. ISRN Chromatography. 2013 Jan 28;2013.
12. Nikita SA, Prashik DB, Madhuri NA. UV-spectrophotometric method development and validation of propranolol hydrochloride and flunarizine dihydrochloride in bulk drug and capsule dosage form. Contemporary investigations and observations in pharmacy. 2012;1:19-23.
13. Dudhe PB, Lahane AV, Borhade KD, Shelke PS, Chavare PD. Spectrophotometric Determination of Acetazolamide in Bulk and Tablet Dosage Form by Area Under Curve and First Order Derivative Methods.
14. Dudhe PB, Shelke P, Chavare P. Determination of Apixaban from Bulk and Tablet Dosage Form by Area Under Curve and First Order Derivative Spectrophotometric Methods. International Journal of ChemTech Research. 2017;10(5):703-11.
15. Komaroju D, Reddy GN, Dhanalakshmi K. Method development and validation for simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumarate in Pure and Tablet Dosage Form by using RP-HPLC. International journal of pharma Research & Review. 2013 Oct;2(10):1-1.
16. Raju NA, Begum S. Simultaneous RP-HPLC method for the estimation of the emtricitabine, tenofovir disoproxil fumerate and efavirenz in tablet dosage forms. Research Journal of Pharmacy and Technology. 2008;1(4):522-5.
17. Raju NA, Rao JV, Prakash KV, Mukkanti K, Srinivasu K. Simultaneous estimation of tenofovirdisoproxil, emtricitabine and efavirenz in tablet dosage form by RP-HPLC. Oriental Journal of Chemistry. 2008;24(2):645-50.
18. Hu M, Wang Q, Ma X, Yang C, Sun H, Liu J, Zhang Y, Xie Y. A Rapid and Sensitive LC Method for Determination of Diastereomeric Purity of Tenofovir Alafenamide. Chromatographia. 2014 Oct 1;77(19-20):1399-1403.
19. Kumar P, Jacob J, Ajina K. Spectroscopic Estimation of Tenofovir Alafenamide, an antiretroviral drug. Research Journal of Pharmacy and Technology. 2016 May 1;9(5):538-540.
20. Khandare B, Dudhe PB, Upasani S, Dhoke M. Spectrophotometric Determination of Vandetanib in Bulk by Area Under Curve and First Order Derivative Methods. International Journal of Pharm Tech Research, 2019, 12(2), 103-110.