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Development and Validation of UV-Vis Spectroscopic Method for the Determination of Anticancer Drug Crizotinib

Mossammat Rima Akter[1], Md. Saddam Hossain[2], Md. Tanvir Hossain Amir[3], Abubakar Sarker[4], Shomrat Saikh[5], Tanvin Sultana[6]

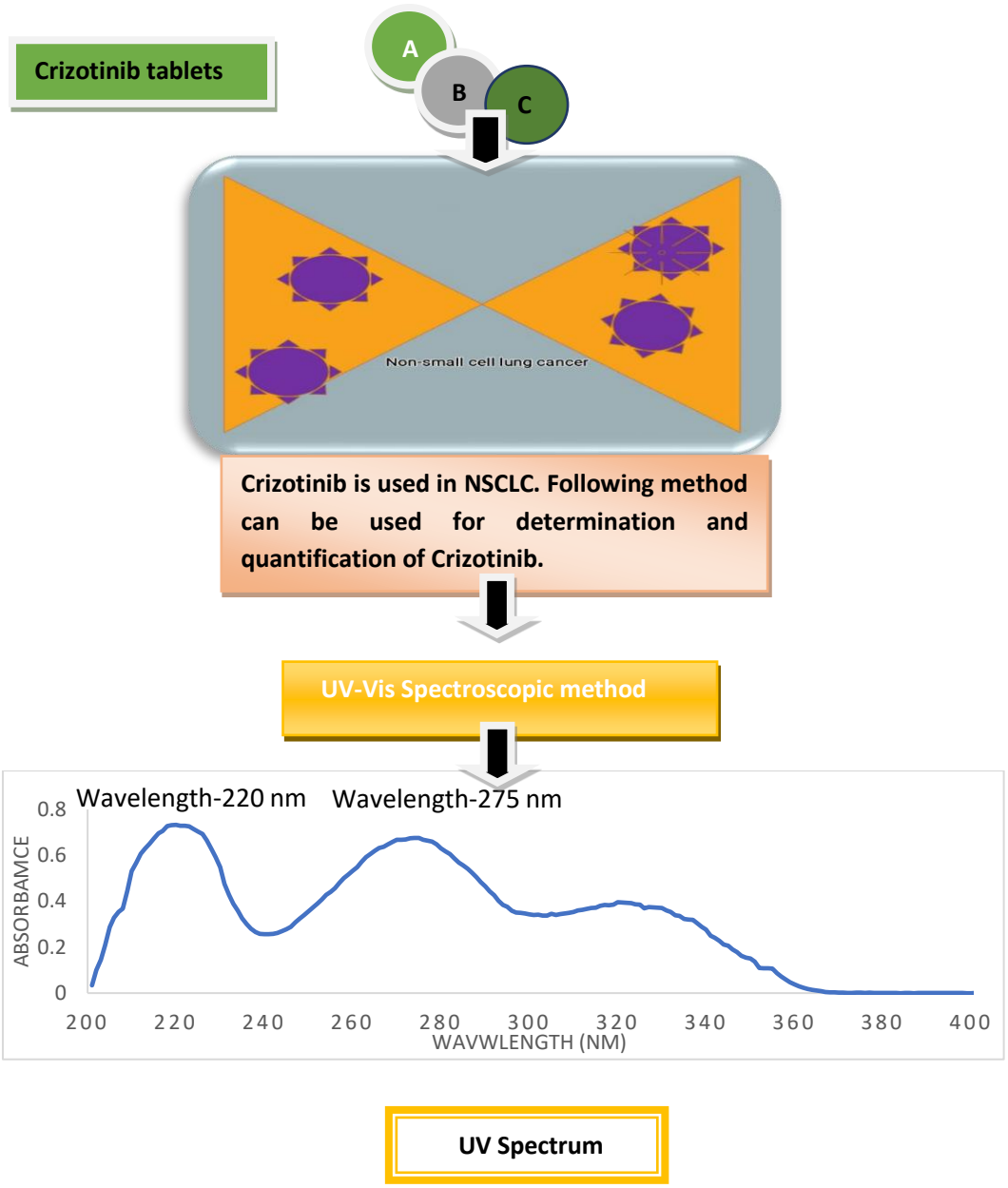
[1,2,5]Masters in Pharmaceutical Science, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[3,4]Bachelor's of Pharmacy, Department of Pharmacy, Southeast University, Dhaka, Bangladesh.
[6]Bachelor's of Pharmacy, Department of Pharmacy, Daffodil International University, Dhaka, Bangladesh.

Abstract

Cancer is one of the most leading causes of death in the recent era. A number of cancers based on the affected organ namely stomach cancer, lung cancer, breast cancer, colon cancer etc. Various anticancer drugs are available worldwide where the topnotch one is tyrosine kinase inhibitor. Crizotinib is the recent INN drug which is a tyrosine kinase inhibitor and indicated for non-small cell lung cancer. It is revealed from literature review, there are no developed methods available for determination of crizotinib except HPLC and UPLC methods. In the present study, authors aimed to develop and validate the method based on UV-vis spectroscopy for determination of crizotinib. Crizotinib showed wavelength max (λ_{max}**)** at 220 nm and 275 nm with a regression equation of y = 0.075x + 0.001, the correlation of Determination (R^2) was 0.999, and the correlation coefficient (r) was 0.9995 which indicate the outstanding correlation between concentrations and absorbances along with linearity of the method. The dynamic range was found 1.25 µg/mL to 20 µg/mL for the method. The % RSD of robustness, precision, ruggedness was below 2. Limit of detection and limit of quantification were detected 0.264 µg/mL and 0.8 µg/mL respectively. This paper introduces a new accurate, precise, and simple method for the analysis of crizotinib. The newly developed method can be used for routine quality control of crizotinib.

Keywords: Method, Validation, Crizotinib, Anticancer drug, Accuracy, Linearity

GRAPHICAL ABSTRACT



1. Introduction

World is getting advanced and modern day by day. Moreover, the modern life is becoming very risky, disease amenable and injurious due to unhealthy lifestyle. This unhealthy lifestyle can be the main culprit of various deleterious diseases including cancer. Cancer refers to a life-threatening condition which is the result of uncontrolled growth and spread of abnormal cell in body [1]. There are various types of cancer based on the origin of cancer namely lung cancer, colon cancer, liver cancer, stomach cancer and bladder cancer etc. The remarkable triggers viz. dietary factors, environment pollutions, certain infections, less physical exercise, obesity etc. are responsible for inducing cancer and increase the risk of cancer [2]. Cancer can be prevented by maintaining balanced and healthy lifestyle and by avoiding cancer triggers as well [2]. The worldwide used anticancer treatments are surgery, radiation therapy, chemotherapy, immunotherapy, hormone therapy, targeted therapy, stem cell therapy etc. Additionally, the familiar

anticancer agents include alkylating agents, antibiotics, topoisomerase inhibitors, antimetabolites, tyrosine kinase inhibitors and mitotic inhibitors [3]. Crizotinib, an anaplastic lymphoma kinase (tyrosine kinase) inhibitor is used in various cancer treatments. Rearrangement of the anaplastic lymphoma kinase (ALK) gene is responsible for 5% of non-small cell lung cancers (NSCLCs), in which a distinct subtype of lung tumor shows exquisite sensitivity to therapy with ALK tyrosine kinase-inhibitors (ALK-TKIs) [4]. Crizotinib has shown preclinical activity, with consistent clinical efficacy in patients with resistant to EGFR inhibitors [5]. Crizotinib was the first targeted therapy discovered to be used as first line treatment of non-small cell lung cancer [6] and also effective in c-Met-amplified NSCLC [7].

Selective and specific methods need to be developed and validated for the determination of crizotinib. Method development means to develop a specific method while method validation ensures that the method is completely developed and suitable for its intended use. Howsoever, literature review revealed that no method has yet been developed and reported on UV-vis spectroscopy although this technique is widely used in pharmaceutical analysis for various drugs. Therefore, the authors adopted interest to develop method based on UV-vis spectroscopy for the quantification of crizotinib. The developed method will be subsidiary for other researchers and manufacturers who are working and interested in the respected field.

2.Literature review

Comprehensive literature review using Goggle Scholar, PubMed, internet, and Sci-hub was done. Only three articles were cited in the literature for the determination of crizotinib, but they were on the development and validation of RP-UPLC (RP-Ultra Performance Liquid Chromatography) method [8] and RP-HPLC method [9, 10] for determination of crizotinib. UV-vis spectroscopic method [11, 12, 13, 14, 15] is comparatively more available, simple and widely used but was not developed and reported yet for the analysis of crizotinib. Therefore, authors of the present study aimed to develop and validate UV-vis spectroscopic method for the analysis of crizotinib. Literature review showed, ascertaining of wavelength max, determination of calibration curve are the integral parts of method development whereas any method must comply with several parameters for being validated as per ICH guidelines [16-31]. The parameters include linearity, accuracy, precision, ruggedness, robustness, specificity, selectivity etc.

3. and Objectives

Crizotinib is indicated for the treatment of non-small cell lung cancer. Literature review revealed clearly that no UV-vis spectroscopic method is available in the literature for the quantification of crizotinib, which is relatively less expensive, and simple. Therefore, the aims and objectives of the present research work are to develop and validate a UV-vis spectroscopic method for the analysis of crizotinib.

4.Experimental

4.1.Reagents & Solvents

HPLC grade Ethanol (CARLO ERBA), Distilled water, HPLC grade methanol (Merck), HPLC grade Acetonitrile (Chemsavers), reagent grade HCl, HPLC grade DMSO (WOLDO) were the required reagents purchased from local vendor.

4.2.Reference standard and Samples

Crizotinib reference standard (100 %), crizotinib raw material, Crizotinib 250 mg tablet were used in the present study.

4.3.Materials

The required materials and glassware included 100 mL volumetric flasks (15), 25 mL volumetric flask,

100 mL volumetric flask, pipette (5, 10, and 20 mL), 100 mL beaker, Aluminum foil, Parafilm foil, Filter paper, Dropper, Test tubes, Slide, Measuring cylinder (100 mL), Sample tube.

4.4.Apparatus

The necessary apparatus used in the present study were Specord Spectrophotometer (205) with a pair of quartz cell (1 cm pathlength) Shimadzu UV Spectrophotometer (UV-1601PC) with a pair of quartz cell (1 cm pathlength), pH meter (HANNA, Portugal).

5.Method Development

5.1.Solubility test

To develop a method for Crizotinib, its solubility was a matter of concern to pick up a suitable solvent. So, several solvents were screened to observe the solubility of crizotinib. Steps of each solubility test are given below:

- 0.1 g crizotinib was weighted and taken in a test tube,
- 1 mL of particular solvent was added in small portions,
- The test tube was shaken after addition of each portion of solvent,
- A homogenous mixture was found.

It was evolved that crizotinib was soluble in methanol, ethanol (warm), DMSO (warm), acetonitrile and mixture of methanol-water (75%:25%).

5.2.Selection of Solvent

Several solvents namely methanol, ethanol, acetonitrile, DMSO were used to test the solubility of crizotinib raw material. Acetonitrile and DMSO are costly solvent. Additionally, heat is required for crizotinib to be dissolved in ethanol. Crizotinib is fairly soluble in methanol. Moreover, methanol is comparatively cheaper and available. As a result, authors selected methanol as a solvent to develop a UV-vis spectroscopic method to detect and quantify of crizotinib.

5.3.Determination of wavelength max (λ_{max})

The standard solution (20 µg/mL) was scanned at 200 to 800 nm and the absorption spectra were recorded. Two absorption bands were observed at 220 nm and 275 nm (Figure 2). It is the first reported spectrum of crizotinib in methanol and it gave the basic information for the development of UV-vis spectroscopic method for the detection and quantification of crizotinib.

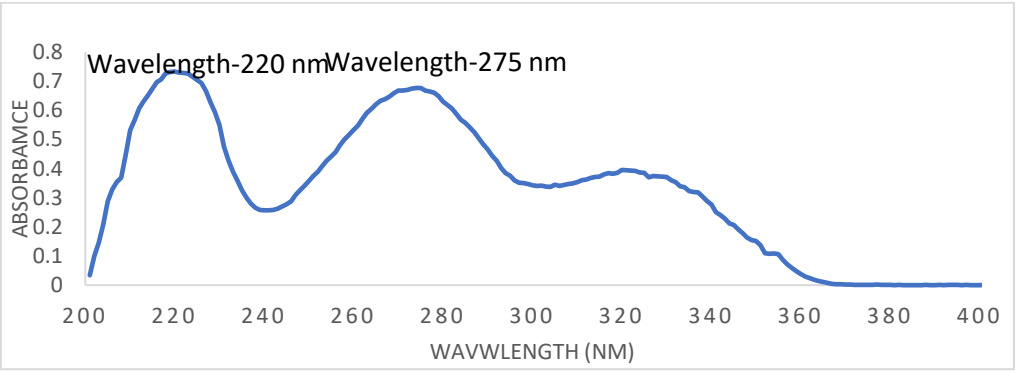


Figure 2: UV Spectrum of Crizotinib (10 µg/mL in methanol)

5.4.Calibration Curve with Crizotinib

Firstly, 0.004 g reference standard of crizotinib (powder) was taken in a 100 mL volumetric flask. Then,

small quantity of methanol was added to the flask, and the flask was shaken rigorously. Then methanol was added up to the mark for making the concentration of the solution 40 µg/mL. It was the stock solution. Later, a serial half dilution was conducted with the stock solution (40 µg/mL) of the crizotinib reference standard to make five diluted reference standard solutions (20, 10, 5, 2.5 and 1.25 µg/mL) for getting regression analysis/calibration curve. The absorbances of the diluted solutions were assessed separately at 220 nm and the data were recorded.

The concentrations of five diluted reference standard solutions were arrayed on X-axis and the recorded absorbances of the respective solutions at 220 nm were portrayed on Y-axis to prepare the mentioned curve (Figure 3).

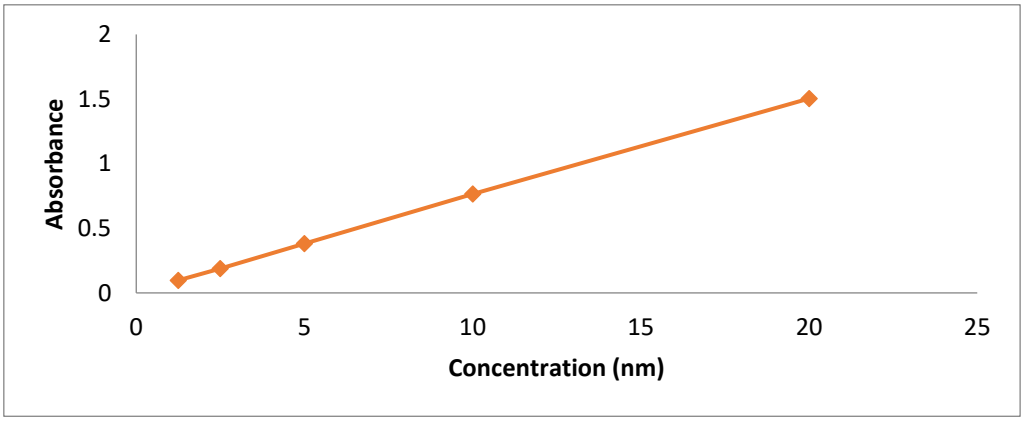


Figure 3: Calibration Curve with Crizotinib Standard Using UV-vis Spectroscopic Method

6.Method Validation:

Validation is the next mandatory step after developing a new method to ensure that the method is completely developed. Therefore, the developed method in the present research for the determination of crizotinib was subjected for validation as per ICH guidelines.

All of the validation parameters which were evaluated for the developed UV-vis spectroscopic method are narrated below:

6.1.Linearity

Stock solution having concentration 40 µg/mL was half diluted to make various solutions of different concentrations such as 20, 10, 5, 2.5 and 1.25 µg/mL.

The concentration of the five diluted standard solutions were portrayed on X-axis and the absorbance of the respective standard solutions at 220 nm were plotted (Figure 3) on Y-axis to figure out a regression equation with coefficient of determination (R^2), calibration curve, and correlation coefficient (r).

6.2.Dynamic range

Dynamic range was estimating by auditing the upper concentration and the lower concentration up to which linearity was observed properly in the calibration curve (Figure 3).

6.3.Specificity/ Selectivity

In a solution of Crizotinib having concentration 10 µg/mL, small amount of magnesium stearate, starch, lactose, were added which are the possible interfering materials used in tablet dosage form. The specificity of UV-vis spectroscopic method was evaluated by computing percentage recovery of crizotinib. The results are presented in the results and discussion section of this paper.

6.4.Intraday precision / Repeatability

Repeatability was resoluted by assaying six replicates of each of the three concentration levels 10µg/mL, 5 µg/mL, and 2.5 µg/mL under the same experimental condition, on the same day. Repeatability was represented by % RSD.

6.5.Inter day precision / Intermediate Precision

Intermediate precision was evaluated by assaying the three replicates of each of the three concentration levels 20µg/mL, 10 µg/mL, 5 µg/mL on three different days, under the same experimental conditions. Precision was notified by % RSD.

6.6.Accuracy

Recovery study help to evaluate the accuracy of UV-vis spectroscopic method. In three solutions of crizotinib having concentration 5 µg/mL, appropriate quantities of crizotinib were further added to each solution making the final concentration of solutions 10 µg/mL, 15 µg/mL, and 20 µg/mL. The accuracy was manifested as percentage recovery of drug. The results are presented in the results and discussions part.

6.7.Ruggedness

Three concentration levels 20µg/mL, 10 µg/mL, 5 µg/mL were assayed in order to evaluating ruggedness of UV-vis spectroscopic method on a separate day by different analyst and device (by using Shimadzu UV-vis spectrophotometer and Specord UV-vis spectrophotometer). Ruggedness was expressed as % RSD.

6.8.Robustness

The influence of small but deliberate change in temperature (by providing mild heat, ± 5^{o}C), pH (by addition of small amount dilute HCl), wavelength (by evaluating at 218, 219, 220 nm), and solvent (methanol, combination of methanol and distilled water) were evaluated to estimate the robustness of UV-vis spectroscopic method. The robustness was presented by %RSD.

6.9.Limit of Detection

The data from calibration curve were analyzed to calculate the standard deviation and slope. Limit of detection was calculated by using following formula:

LOD = (3.3* Standard deviation of intercept)/Slope

6.10. Limit of Quantification

Standard deviation and slope were calculated by analyzing the data from calibration curve. Limit of quantification was calculated by using the defined formula:

LOQ = (10*Standard deviation of intercept)/Slope

7. Results and Discussion

7.1.Linearity

The plot of concentration vs. absorbance of five diluted standard solutions of crizotinib is shown below (Fig.3). The calibration curve has the regression equation y = 0.075x + 0.001, coefficient of determination (R^2 = 0.999) and correlation coefficient (r= 0.9995). Regression analysis results were presented in the Table 1. Coefficient of determination value indicates outstanding data fit and linearity of the calibration curve of the UV-Vis spectroscopic method developed under the present work. Correlation coefficient value proved a perfect correlation existing between concentrations and absorbances. Similar result was obtained in UV-vis spectroscopic method with osimertinib [19].

Table 1: Results of Regression Analysis for UV-vis Spectroscopic Method	
Linear or Dynamic range	**1.25-20 µg/mL**
Slope	**0.075**
Intercept	**0.001**
Coefficient of determination	**0.999**
Correlation coefficient	**0.9995**

7.2.Dynamic range

Dynamic range for UV-vis spectroscopic method was found 1.25 µg/mL to 20 µg/mL (Fig.3). In the mentioned range, precision, and accuracy were evaluated following ICH guidelines [16, 17, and 18]. The estimated results were depicted in the (Table 3, 4, 5 and 7). It is conspicuous from the (Table 3, 4, 5 and 7) that % RSD value did not exceed the ICH guideline value 2%. Therefore, the developed method can be applied for the quantification of crizotinib in the bulk and formulations with the appropriate precession and accuracy.

7.3.Specificity/ Selectivity

The percentage recovery of drug was obtained 99.7 % – 103.31 % (Table 2) in presence of small quantities of magnesium stearate, starch, lactose. Percentage recovery of crizotinib was calculated by using a formula, (recovered amount/ added amount) *100%. Based on the obtained result, it is obvious that the analyte was almost completely interaction free from the excipients. The results alluded the specificity of the developed method for the detection and estimation of crizotinib from the dosage form and the bulk.

Table 2: Results of Selectivity/ Specificity Test Using UV-vis Spectroscopy		
Concentration added ((µg/mL))	**Recovered Amount (µg/mL)**	**% Recovery**
10	10.26	103.31
10	9.97	99.7
10	10.13	101.3
Standard Deviation	± 0.145	± 1.81
Mean	10.12	101.4
% RSD	1.44	1.78

7.4.Intraday precision / Repeatability

Percent (%) RSD for 6 replicates of crizotinib solution having concentration 10 µg/mL was 0.6689 (Table 3), percent (%) RSD for 6 replicates of crizotinib solution having concentration 5 µg/mL was 1.23 (Table 4) and percent (%) RSD for 6 replicates of crizotinib solution having concentration 2.5 µg/mL was 0.887 (Table 5). All % RSD values were less than 2 concluding excellent precision of results obtained by the developed method.

Table 3: Results of Repeatability Test 1 Using UV-vis Spectroscopy

Sample number	Concentration ((µg/mL))	Absorbance
1	10	0.7642
2	10	0.7583
3	10	0.7725
4	10	0.7637
5	10	0.7706
6	10	0.7662
Standard deviation		**0.0051**
Mean		**0.7659**
% RSD		**0.6689**

Table 4: Results of Repeatability Test 2 Using UV-vis Spectroscopy

Sample number	Concentration (µg/mL)	Absorbance
1	5	0.3813
2	5	0.3778
3	5	0.3761
4	5	0.3692
5	5	0.3822
6	5	0.3778
Standard deviation		**0.0046**
Mean		**0.3774**
% RSD		**1.23**

Table 5: Results of Repeatability Test 3 Using UV-vis Spectroscopy

Sample number	Concentration((µg/mL)	Absorbance
1	2.5	0.1873
2	2.5	0.1903
3	2.5	0.1892
4	2.5	0.1913
5	2.5	0.1921
6	2.5	0.1897
Standard deviation		0.0017
Mean		0.189
% RSD		0.887

7.5.Inter day precision / Intermediate Precision

Percent (%) RSD for crizotinib solution having concentration 10 µg/mL tested on three different days was 0.752 (Table 6), % RSD for crizotinib solution having concentration 5 µg/mL tested on the three different days was 0.442 (Table 6) and % RSD for crizotinib solution having concentration 2.5 µg/mL tested on the

three different days was 0.265 (Table 6). All evaluated values of % RSD were less than 2 concluding faithful and clean intermediate precision of the results acquired by the newly developed method.

Table 6: Results of Inter day repeatability Test Using UV-vis Spectroscopy

Conc. (µg)	Average absorbance in Day 1	Average absorbance in Day 2	Average absorbance in Day 3	Standard Deviation	Mean	%RSD
20	1.5063	1.4911	1.5132	0.011	1.504	0.752
10	0.7642	0.7614	0.7575	0.003	0.761	0.442
5	0.3813	0.3793	0.3801	0.001	0.380	0.265

7.6.Accuracy

The accuracy of the UV-vis spectroscopic method developed in the present study was confirmed by recovery studies. The percentage recovery was from 99.67 to 102.5 % (Table 7) which ensured appreciable accuracy of the results attained by the developed method. So, the method was proved as accurate for the quantification of Crizotinib.

Table 7: Results of Accuracy Test Using UV-vis Spectroscopy

Previous concentration (µg/mL)	Amount added (µg/mL)	Amount Found (µg/mL)	% Recovery
5	5	9.97	99.7
5	10	14.95	99.67
5	15	20.05	102.5

7.7.Ruggedness

Percent (%) RSD of crizotinib solutions having concentration 20 µg/mL, 10 µg/mL, 5 µg/mL was observed 0.339, 0.436, and 0.223 respectively (Table 8). The low % RSD ascertained adequate ruggedness of the developed method.

Table 8: Results of Ruggedness Test Using UV-vis Spectroscopy

Conc. (µg/mL)	Average absorbance in Lab 1	Average absorbance in Lab 2	Standard Deviation	Mean	%RSD
20	1.5063	1.4991	0.005	1.5027	0.339
10	0.7642	0.7595	0.003	0.7619	0.436
5	0.3813	0.3801	0.001	0.3807	0.223

7.8.Robustness

There was no evidence of significant change in absorbance of crizotinib after studious changing of temperature, pH, solvent as well as wavelength. Low % RSD values vindicated the strong robustness of the newly developed method (Table 9).

Table 9: Results of Robustness Test Using UV-vis Spectroscopy		
Parameters		**% RSD**
Temperature	**Normal Condition (25 º C)**	**1.2**
	Temperature change (± 5 º C)	**1.6**
pH	**Normal Condition**	**1.5**
	pH change (± 0.5 unit)	**1.9**
Wavelength (nm)	**218**	**1.51**
	219	**1.02**
	220	**1.3**
Solvent	**Methanol**	**1.1**
	(Methanol + distilled water)	**1.21**

7.9.Limit of Detection

Limit of detection of crizotinib was evaluated as stated in the method validation section (6.8) and was found 0.264 µg/mL which is the lowest concentration of crizotinib that can be detected by the developed method.

7.10. Limit of Quantification

Limit of quantification was evaluated as stated in the method validation section (6.9) and was found 0.8 µg/mL which is the lowest concentration of crizotinib that can be quantified by the developed method.

8. Application of the developed method to assay crizotinib in Tablet:

The newly developed method was used for the quantification of crizotinib in a tablet dosage form (250 mg) of a renowned pharmaceutical company. The result showed (Table 10) percentage recoveries were high (99.99-100.05) and % RSD values were low (0.22), which confirms the competency of the developed method for diurnal determination of crizotinib in pharmaceutical preparations.

Table 10: Results of Tablet (250 mg) assay using UV-Vis Spectroscopic method		
Concentration added	Recovered Amount (Microgram)	% Recovery
250 mg	248.9	99.56
250 mg	249.8	99.92
250 mg	249.96	99.98
Standard Deviation	0.571430952	0.227156334
Mean	249.5533333	99.82
%RSD	0.228981495	0.227565953

9. Conclusion

The present research work was accomplished with the development and validation of a UV-vis spectroscopic method for the detection and quantification of crizotinib. All ICH guidelines were maintained and followed to effectively vindicate the method for analysis of crizotinib in raw material and pharmaceutical dosage forms. The test of crizotinib tablet by using the newly developed method confirms the compatibility and suitability of the method for pharmaceutical dosage form. The developed method was quite accurate, simple, precise, rapid, sensitive, specific, robust and rugged for determination and quantification of crizotinib. The established method is immensely delegated for using in quality control of crizotinib at any quality control laboratory, quality assurance laboratory and any biopharmaceutics and bioequivalence laboratory as well.

10. Funding

Authors conducted the present study with self-fund.

11. Conflict of interest

The authors have no conflict of interest.

12. Acknowledgement

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13. Ethics statement

This submission has followed the journal Ethical Guidelines.

14. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

15. Declaration of interests

Please **tick** the appropriate statement below (please do not delete either statement) and declare any financial interests/personal relationships which may affect your work in the box below.

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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