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Original Article

DEVELOPMENT AND VALIDATION OF NOVEL UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF TEPOTINIB IN BULK AND IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: The objective of the study is to develop a new, simple, rapid, accurate, and economical UV-spectrophotometric method and validate the same for the estimation of Tepotinib in bulk and in pharmaceutical formulation as per ICH guidelines.

Methods: The present work was carried out using the UV-Visible double-beam spectrophotometer model Systronics 2201. Tepotinib was found soluble in water, methanol, methanol so, analytical-grade methanol was used as a solvent for conducting the work. The λ_{max} of the tepotinib was determined by dissolving pure drug in methanol scanned in the range of 200-800 nm. The present method was validated for the linearity, accuracy, precision, Limit of Detection and Limit of Quantification.

Results: The maximum absorbance of tepotinib obtained at a wavelength of 272 nm. The method was found linear in the range of 3-15 µg/ml with the regression coefficient of 0.996 and the equation y = 0.0699x+0.0335. The accuracy was found to be in the range of 96.8-98.5%, the intra-day and inter-day precision % RSD value was 0.262 and 0.69, respectively and the LOD and LOQ were 0.0925 µg/ml and 0.28 µg/ml respectively.

Conclusion: The method demonstrated good reproducibility and recovery so, proposed method can be successfully applied for the routine analysis of tepotinib in bulk and pharmaceutical dosage form.

Keywords: Tepotinib, UV spectrophotometer, Methanol, Validation, International council for Harmonization guidelines

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INTRODUCTION

For the treatment of metastatic non-small cell lung cancer in individuals with MET exon 14 skipping mutations, tepotinib is an oral tyrosine kinase inhibitor. Tepotinib is known by its chemical name 3-{1-[3-[5-[(1-methyLpiperidin-4-yl) methoxy] pyrimidin-2-yl] phenyl] methyl]-6-oxopyridazin-3-yl} benzonitrile [1]. Fig. 1 gives structure of Tepotinib. It has a 492 g/mol molecular weight. Its chemical structure is C29H28N6O2. Tepotinib structure is shown in fig. 1. Tepotinib is a MET tyrosine kinase inhibitor used to treat various solid tumours that overexpress MET. Tepotinib preferentially binds to MET tyrosine kinase and blocks MET signalling pathways, which may cause tumour cells that overexpress this kinase to undergo apoptosis [2]. Tepotinib is soluble in acetonitrile, water, methanol, and ethanol. As a result, methanol was used as a diluent for the job.

Literature survey reveals that only few methods are available for the determination of tepotinib, such as RP-HPLC [3, 4], spectrofluorimetric methods [5] as a combination with other drugs of the same class and LC-MS/MS methods [6]. However, according to the knowledge of the authors, no spectroscopic method was developed for the estimation of tepotinib in bulk and formulation. Hence, in the present work, an experiment was made to develop a simple, sensitive and economical UV-Visible spectrophotometric method for the analysis of Tepotinib in bulk and formulation according to ICH guidelines [7].

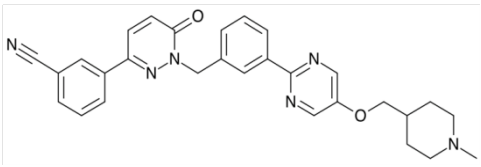

Fig. 1: Structure of tepotinib

MATERIALS AND METHODS

Instrumentation

UV-visible double beam spectrophotometer (Systronics 2201) was used for conducting the work. Analytical balance (Shimadzu AY220) was used for weighing and Ultra Sonicator (Oscar Microclean 103) was used to sonicate the standard and product sample solution [8].

Chemicals and reagents

Tepotinib (API) was procured from Spectrum Pharma Research Solutions Private Limited, Hyderabad. Tablets of Tepotinib were purchased from India mart app. Methanol, Ethanol, Acetonitrile were of analytical standard and purchased from Merck Life sciences Private Limited.

Selection of solvent

Tepotinib is dissolved in various solvents to select the solvent of good solvability. Various solvents i.e., methanol, ethanol, acetonitrile. Hence, the Tepotinib was soluble in methanol and showed maximum absorbance at 272 nm [8].

Preparation of standard drug solution

10 mg of Tepotinib was weighed and transferred to 10 ml volumetric flask and dilute up to the mark with methanol (1000 µg/ml). Pipette out 1 ml from stock solution and dilute it to 10 ml with methanol (100 µg/ml) [8].

Working standard solution

1 ml of the standard stock solution was transferred into 10 ml volumetric flask, 5 ml of methanol was added and volume was made up to the mark with methanol. (Concentration of Tepotinib: 10µg/ml) [9].

Preparation of sample solution

Preparation of sample solution: Weigh accurately and finely powdered 10 tablets. Transfer the powder equivalent to 10 mg of

tepotinib into 10 ml volumetric flask. Then added 5 ml of methanol and sonicate for 10 min, volume was made up to the mark with methanol (1000 µg/ml). Pipette out 0.1 ml of the above solution in 10 ml volumetric flask, added 5 ml of methanol and volume was made up to the mark with the same (concentration of Tepotinib: 10 µg/ml) [9].

Determination of wavelength of maximum absorbance (λ_{max})

The working standard solution (Concentration of Tepotinib: 100 µg/ml) was scanned utilizing full scan mode with medium scanning speed for the whole range of UV-VIS spectrophotometers, ranging from 200-800 nm with methanol as blank. After acquiring the spectrum, λ_{max} was identified at 272 nm [10].

RESULTS

Method development

The developed method was validated as per ICH guidelines. The parameters assessed were linearity, range, accuracy, precision (repeatability), Limit of Detection and Limit of Quantification [9-12].

Linearity and range

Linearity is defined as an ability of the analytical procedure to obtain test results which are directly proportional to the concentration of the analyte in the sample. Pipette out 0.3, 0.6, 0.9, 1.2, and 1.5 ml from the solution of 100 µg/ml and dilute to 10 ml with the methanol. Concentration is 3,6,9,12,15 µg/ml. Taking the absorbance at 272 nm and calculate regression coefficient for the range of (3-15 µg/ml) [9].

Accuracy

Accuracy is defined as, analytical procedure it expresses the closeness of an agreement between the value that is accepted and either as a true conventional value. Accuracy was calculated at three levels; these are 80%, 100%, 120% by standard addition method. A known amount of standard Tepotinib solution (API) was spiked to the tablet solution [10]. The % recovery was calculated by

$$\% \text{ Recovery} = \text{Observed value} \div \text{True value} \times 100.$$

Precision

Precision is defined as an analytical procedure is to define the closeness of agreement between a sample of measurements obtained from multiple sampling of the same homogenous sampling in specific conditions. Precision was determined by taking six readings of 9 µg/ml concentration intraday and inter-day. The relative standard deviation (%RSD) was calculated [11].

Limit of detection (LOD)

Limit of detection is defined as the lowest amount of analyte in a sample that can be detected. LOD is based on the standard deviation value from precision and slope of the regression coefficient. The formula for calculating LOD is $LOD = 3.3 \times SD \div S$ [11].

Where SD= Standard deviation, S = Slope of regression coefficient.

Limit of quantification (LOQ)

Limit of quantification is defined as the lowest amount of analyte in the sample that can be quantified. LOQ is calculated by the formula; $LOQ = 10 \times SD \div S$ [11].

Where SD = Standard deviation, S = Slope of regression coefficient.

Robustness

Robustness is defined as, the capacity of an analytical procedure to remain unaffected by small changes or deliberate variation in the method parameters. Aim of this study is to validate the method that allow for small variations in parameters. Here, the wavelength was changed to 256 nm and 258 nm [12].

Ruggedness

Ruggedness is defined as, the reproducibility of the results when the defined method is performed under different analysts, laboratories, and instruments [12].

Method validation

Selection of detection wavelength (λ_{max})

Tepotinib showed maximum absorbance at 272 nm and was selected as detection wavelength as shown in fig. 2.

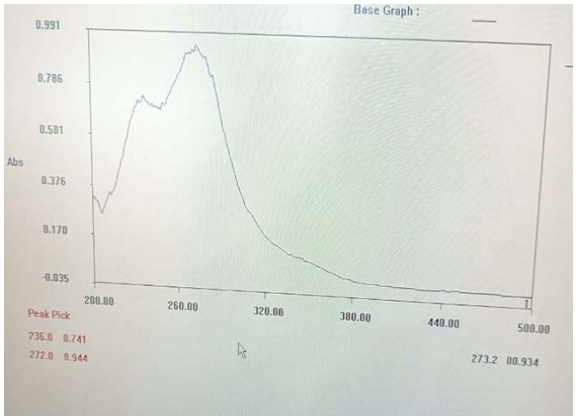

Fig. 2: Scan analysis of tepotinib

Linearity and range

Table 1: Calibration data for tepotinib

Concentration (µg/ml)	Absorbance
3	0.22
6	0.468
9	0.679
12	0.887
15	1.059

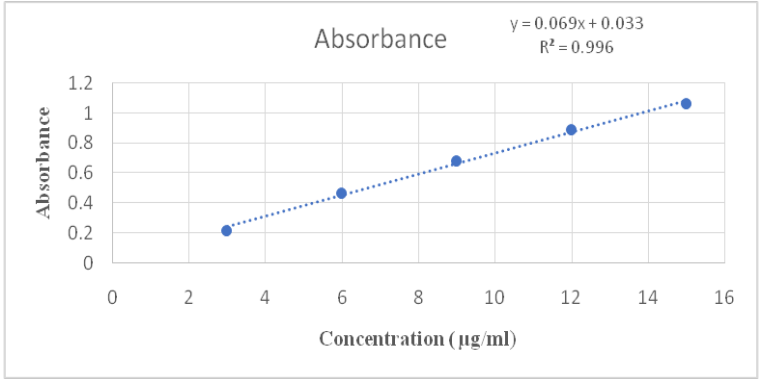

Fig. 3: Graph of calibration curve

The Tepotinib follow the Beer's Lambert Law in the linearity range of 3-15 µg/ml (table 1). The regression coefficient R^2 value is 0.996 with the equation y = 0.0699x+0.0335 (fig. 3). The R^2 value is in the limit, so the linearity parameter is validated.

Accuracy

Table 2: Result of recovery study of tepotinib

S. No.	% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	%Recovery
1	80	8	7.75	96.8
2	100	10	9.79	97.6
3	120	12	11.82	98.5

% Recovery of tepotinib is within limit; hence the accuracy parameter is validated.

Precision

Table 3: Results for Intra-day precision

Readings	Concentration	Absorbance	Statistical analysis
1	9µg/ml	0.747	Mean= 0.748
2		0.747	Standard Deviation= 0.0019
3		0.749	%RSD=0.2627
4		0.752	
5		0.747	
6		0.748	

n= 6 responses, %RSD: Relative standard deviation

Table 4: Results for Inter-day precision of tepotinib

Readings	Concentration	Absorbance		Statistical analysis
		Day-1	Day-2	
1	9µg/ml	0.747	0.65	Day 2-Mean= 0.6438
2		0.747	0.647	Standard deviation=0.0040
3		0.749	0.643	% RSD= 0.690
4		0.752	0.645	
5		0.747	0.64	
6		0.748	0.638	

n= 6, %RSD= Relative standard deviation, The %RSD value for intra-day and inter-day precision is<2% (0.262 and 0.69 respectively), which is in the limit hence the precision parameter is validated.

Limit of detection (LOD)

The LOD for Tepotinib was found to be 0.0925 µg/ml. Sensitivity parameter is validated.

Limit of quantification (LOQ)

The LOQ for Tepotinib was found to be 0.28 µg/ml. Sensitivity parameter is validated.

Robustness

Table 5: Result of robustness

Wavelength	Absorbance	Average	Statistical analysis
256 **nm**	1.374	1.374	Standard Deviation= 0.001
	1.375		% RSD= 0.7278
	1.373		
258 **nm**	1.375	1.375667	Standard Deviation=0.00115
	1.377		% RSD= 0.7278
	1.375		

n= 3, %RSD= Relative standard deviation, The robustness for change in wavelength was observed and the %RSD value is within the limit i.e.,<2%. Hence the robust parameter is validated.

Ruggedness

Table 6: Result of ruggedness

Concentration	Analyst	Absorbance	Statistical Analysis
12 (µg/ml)	1	0.885	Mean 0.886
		0.886	SD 0.001
		0.887	%RSD 0.1128
Concentration		Absorbance	Statistical Analysis
12 (µg/ml)	2	0.886	Mean 0.887
		0.887	SD 0.001
		0.888	%RSD 0.1127

n= 3, % RSD= Relative standard deviation, Ruggedness was observed by taking two different analyst and measured the absorbance for 12 µg/ml concentration. The %RSD found for Analyst 1 and 2 was 0.1128 and 0.1127, respectively. The value is in limit so ruggedness parameter is validated.

DISCUSSION

A UV-VIS spectrophotometric method was developed and validated for the estimation of tepotinib in bulk and formulation as per ICH guidelines. The solvent used was analytical-grade methanol. The absorbance was noted at 272 nm. The spectrum was shown in fig. 3. The absorbance of tepotinib at 272 nm were measured and calibration curve for different concentration of tepotinib were plotted. From the calibration curve, the data for the regression equation was found to be y= 0.0699+0.0335 and the correlation coefficient was noted as R2= 0.996. Linearity was found to be within the concentration 3-15µg/ml. The accuracy of the method was calculated by percentage recovery studies. Percentage recovery was found to be 96.8-98.5. The results were within the limit. Hence, the method was found to be accurate. The precision of the method was studied by repeatability. %RSD for intraday precision and inter-day precision was found to be 0.2627 and 0.69, respectively. The %RSD values for ruggedness were found to 0.1128 and 0.1127, which was within the limits. Hence, the method was found to be rugged. The robustness results of the method were performed by deliberate change in the detection wavelength. %RSD was found to be 0.07278, which was within the limits. Hence, the proposed method was found to be robust. On the premise of wavelength, diluents, stability study, limits, accuracy, precision, and application of the UV methods, a comparison of previously published works and recently performed work was observed and carried out. The comparison details are shown in table 7.

Table 7: Comparison between previously published UV methods

S. No.	Diluent	Wavelength	Applications	Reference
1	-	285 nm	Drug content analysis from tablets and nanoparticles preparations. Additionally, the method was successfully employed for pH metric solubility analysis of the drug.	[14]
2	Phosphate Buffer solution	228 nm	Developed with the two methods zero-order (method I) and the zero-order AUC (method II).	[20]
3	Acetonitrile	315 nm	Good reproducibility and recovery.	[21]
4	40% solvent A, 20% methanol, and 40% acetonitrile. Solvent A consisted of water (72.5%), methanol, (25%), and triethylamine (2.5%)	267 nm	Sensitivity of this new method is sufficient to perform therapeutic monitoring and pharmacokinetic studies.	[9]

There is no UV spectroscopic method that can be used for variety of analysis of tepotinib. When compared to previously developed works of similar nature and the current method; which uses methanol as diluent at detection wavelength 272 nm is shown to be more accurate, precise, economic, cost-effective, simple.

CONCLUSION

The UV spectrophotometric method was developed for the estimation and quantification of Tepotinib as per ICH guidelines using methanol as solvent. There is no previous any work on UV-VIS spectrophotometric method is available for the estimation of tepotinib. The proposed method was found to be advantageous because of rapid analysis, cost-effectiveness, easy preparation of sample, good reproducibility of results, accurate, precise, economic and practical. Hence, the proposed method can be used for routine quantification of tepotinib in bulk and formulation**.**

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AUTHORS CONTRIBUTIONS

For conducting the research work, all the authors have contributed equally. Shubhangi Birajdar gave her contribution by collection of chemicals and reagents and in experimental work in our laboratories required for conducting the necessary work. Dr. Smita Kumbhar gave contribution by interpreting and analysing the data. Mallinath Kalshetti gave support by conducting the work under his guidance. Monika Shirawar drafted the manuscript and revised it for quality manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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