Research Article

Development and Validation of Liquid Chromatography Method for Determination of Tepotinib in Bulk Drug and in Tablet Dosage Form

Hameeda Begum*, Mohammed Arsalan, Madiha Jabeen
Student, Sultan-ul-uloom college of Pharmacy, Hyderabad 500034, Telanagana, India.
***Corresponding author's E-mail:** Hameedabegumus.456@gmail.com

Received: 10-03-2023; **Revised:** 20-05-2023; **Accepted:** 26-05-2023; **Published on:** 15-06-2023.

ABSTRACT

The development and validation of an accurate, sensitive, precise, rapid, and isocratic reversed-phase HPLC (RP-HPLC) method for simultaneous estimation of Tepotinib in bulk drug and Pharmaceutical fixed dosage forms has been accomplished. The best separation was achieved on a 250 mm × 4.6 mm i.d., 5-µm particle size of x-tera RP-18 with buffer: Acetonitrile [(40:60) (v/v)] in the isocratic mode of elution as mobile phase at a flow rate of 1 mL min−1. UV detection was at 254 nm. Retention times were found to be 2.832 min for Tepotinib. Response was a linear function of concentration over the range of 45-270 mcg/mL for Tepotinib with correlation coefficient of around 0.9988. The percentage assay of Tepotinib found was found to be 99.37. The limit of detection (LOD) and the limit of quantification (LOQ) for Tepotinib were found to be 0.0225mcg/mL and 0.0675mcg/mL respectively. The excipients in the formulation had no effect on the assay. The method is suitable for use in quality-control laboratories because it is simple, quick, and accurate and precise.

Keywords: Tepotinib, formulation, evaluation, analytical validation.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2023.v80i02.019

DOI link: http://dx.doi.org/10.47583/ijpsrr.2023.v80i02.019

INTRODUCTION

Tepotinib is a kinase inhibitor potential anti-cancer properties. TEPMETKO (tepotinib), a film coating tablets for oral use are formulated with Tepotinib hydrochloride hydrate as active ingredient.[1] Tepotinib which is a kinase inhibitor that targets mesenchymal-epithelial transition factor (MET), including exon 14 skipping variants; it inhibits hepatocyte growth factor (HGF)-dependent and -independent MET phosphorylation and MET-dependent downstream signalling pathways. Tepotinib also inhibited melatonin 2 and imidazoline 1 receptors at clinically achievable concentrations and subsequent downstream signalling pathways to reduce tumour cell proliferation, anchorage-independent growth, and migration of MET-dependent tumour cells. The proto-oncogene c-Met produces the receptor tyrosine kinase MET, also known as the hepatocyte growth factor receptor or HGFR, which is overexpressed or mutated in many different types of tumour cells. This protein is essential for tumour cell survival, invasion, and metastasis as well as tumour angiogenesis. In c-MET amplified gastric cancer cells, tepotinib has also been observed to upregulate the expression of epithelial-mesenchymal transition (EMT)-suppressing genes (such as MUC5AC, MUC6, GSK3, and E-cadherin) while down-regulating the expression of EMT-promoting genes (such as MMP7, COX-2, WNT1, and c-MYC). This finding suggests that the tumor-suppressing activity of that clinically relevant concentrations, it has also been demonstrated to inhibit melatonin 1B and nischarin; however, the significance of this activity in relation to tepotinib's mode of action is unclear.[2]

At the time of the present method's development, no liquid chromatographic methods for the determination of Tepotinib by reversed-phase high-performance liquid chromatography (RP-HPLC) had been described in the literature. However, no methods for simultaneous quantification of Tepotinib in oral fixed dosage form are available in the literature. Furthermore, no official or draft monograph on this analyte drug has been published in any pharmacopoeia for compendial applications. The goal of the research was to create a precise and efficient RP-HPLC method for estimating new anticancer agents in oral fixed dosage forms. This paper also addresses the validation of the developed method in accordance with ICH guidelines 10.[3]

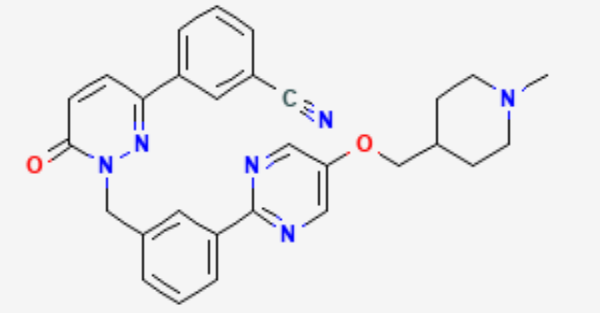

Figure 1: Structure of tepotinib

MATERIALS AND METHODS

Chemicals and Reagents:

Tepotinib 99% pure, Acetonitrile HPLC Grade and Methanol HPLC Grade, 0.03M Potassium dihydrogen phosphate, dilute orthophosphoric acid.

Chromatography Instrument:

A Liquid Chromatrograph is equipped with variable wavelength with UV detector and LC solution software.[4] A X-Terra RP-18, 250mmx4.6mm, 5µ column is used. The column was maintained at isocratic conditions with a flow rate of 1ml/min. The contents of mobile phase were Buffer along with Acetonitrile in the ration of 40:60. The buffer used during the analysis was 0.03M Potassium dihydrogen phosphate in 1000ml of water, adjust the pH 3.0 with dilute orthophosphoric acid. The diluent which was used was water and Acetonitrile in the ration of 50:50. The injection volume was 20µl and UV detection was performed at 254 nm.[5]

Preparation of the Primary Standard Drug solutions: A standard stock solution of the drugs was prepared by dissolving 225 mg of Tepotinib in 100 mL volumetric flask. Dissolve and dilute to volume with diluent (50:50 v/v acetonitrile: water).

Preparation of Working Standard Drug Solution: 5.0 mL of the above stock solution was taken in 50 mL volumetric flask and thereafter made up to 50 mL with diluent (50:50v/v acetonitrile: water) to get the working standard solution

Sample Preparation: A test solution was prepared by Transfering 510 mg of TEPMETKO formulation into a 100ml volumetric flask, dissolve and dilute to volume with diluent (50:50 v/v acetonitrile: water). Take 5ml of the above test solution to 50ml volumetric flask dilute with diluent.

Linearity*:* After preparation of diluent inject the diluent as blank and ensure a steady base line. Later on inject 0.15mg/ml of Tepotinib of standard solution and sample then record the response

Accuracy: The accuracy of the method was determined by measuring the recovery of the drugs by the standard addition method.[6] To find out whether the analytes in the formulation caused positive or negative interferences, known dosages of each drug (10% standard drug solution) were added to a formulation mixture at concentrations of 80%, 100%, and 120% of the target test concentrations. At each level, the same additions were made three times. The process for preparing the extraction sample is followed, and it is measured against a reliable reference standard. The percentage of analytes that the assay successfully covered served as an indicator of accuracy.[7] The findings of accuracy studies from standard solution and process-related impurity are presented in Table-; recovery values showed that the method was accurate within the desired range

Precision: A liquid chromatograph is outfitted with a UV detector, variable wavelength, and the LC solution software. The column used is a X-Terra RP-18, 250mmx4.6mm, 5µ. With a flow rate of 1 ml/min, isocratic conditions were maintained in the column.[8] Buffer and Acetonitrile were the two ingredients in the mobile phase, in a ratio of 40:60. 0.03M Potassium dihydrogen phosphate in 1000ml of water served as the analysis' buffer.[9] Use diluted orthophosphoric acid to bring the pH up to 3.0. Acetonitrile and water were diluted 50:50 as the diluent in use. UV detection was done at 254 nm with a 20l injection volume. Inject the 0.225mg/ml TEPOTINIB standard solution 6 times record the response. Inject the TEPMETKO sample in 6 times and record the response.[10]

Limits of Detection and Quantification: The method's limit of detection (LOD) was established as the lowest concentrations of active pharmaceutical ingredients producing a S/N ratio of approximately 3.[11] The lowest concentrations of active pharmaceutical ingredients that can be quantitated with acceptable accuracy and precision and that produce a signal-to-noise ratio (S/N) of about 10 were identified as the limit of quantification (LOQ).[12]

Method Applicability: Our research team tested the newly developed method by using it to estimate Tepmetko in pharmaceutical film coated tablet.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization:

A gradient reversed-phase HPLC method was developed for the quantification of the aforementioned active pharmaceutical ingredients in response to the lack of an easy, trustworthy, and quick method of analysis for determining Tepmetko concentrations in pharmaceutical matrices. We examined a variety of HPLC method variables in relation to their corresponding effects on the analysis's findings. The active pharmaceutical analyte had a good UV response and was interference-free at 254 nm. The analyte peak was distinct and tailing-free in these circumstances. The set of conditions mentioned earlier in this article was chosen for additional validation after taking into account the entirety of the data obtained from this extensive study.

Method Validation Tests: The following method validation characteristics were investigated: method precision (RSD %), method accuracy (recovery% and RSD,%), linear range (correlation coefficient), and LOD & LOQ.

Linearity: The plot of peak areas of each sample against concentrations was found to be linear for Tepotinib in the range of 45-270 g/mL, with a correlation coefficient of 0.9988. (Table). Table I shows the linear regression least square fit data obtained from the measurements. Tepotinib's linear regression equation is y = 34018x. Table 1 shows the regression characteristics for this method, such as slope, intercept, and %RSD. These findings show a strong relationship between peak areas and analyte concentration.

Accuracy: Recovery of the individual substances at 80%, 100%, and 120% of specified concentrations were 109.6%, 98.0%, 117.0% respectively. which proves the accuracy of the method. From these data, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (Table 2).

Precision: The intra-day and inter-day variability or precision data are summarized in Table 3. The method's repeatability is indicated by the low RSD value (1%). These results show that the method has a high degree of precision and reproducibility, both within and between analytical runs (Table 3).

Limit of Detection and Limit of Quantifications: A signal-to-noise ratio of 10:1 can be used to calculate the LOQ, or it can be approximated by multiplying the LOD by 3.3. This method is commonly used for analytical methods with baseline noise. The limit of detection (LOD) and limit of quantification was found to be 0.01% and 0.03% respectively. These values reflect the method's high sensitivity, which is critical in most studies, as well as the method's ability to detect and quantify analytes over a wide concentration range.

Table 1: Certain parameters of Tepotinib

Parameter	Tepotinib
Retention Time (min)	2.8325
Tailing Factor	1.09
Peak areas	7747133
Percentage of peak areas	99.678
Theoretical Plates	2804.295
Resolution	1.6875
Linear range in (µg/mL)	45-270
Limit of Detection (LOD) (mcg/mL).	0.0225
Limit of Quantification (LOQ) (mcg/mL)	0.0675
Correlation Coefficient (r)	0.9988
% of Assay	99.37%

Table 2: Accuracy study of Tepotinib dilutions

Accuracy/Recovery Study of Tepotinib Dilutions						
S.No	Recovery at 80% dilution Level Peak areas		Recovery at 100% dilution Level Peak areas		Recovery at 120% dilution Level Peak areas	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	5906572	6804782	7670239	8429009	9284253	10331455
2	5937767	6808106	7669595	8424708	9287667	10304172
3	5909404	6802275	7668384	8422411	9305486	10307276
Avg	**5917914**	**6805054**	**7669406**	**8425376**	**9292468.7**	**10314301**
Std.Dev	17251	2925	942	3349	11402	14937
%RSD	0.292	0.043	0.012	0.040	0.123	0.145
Recovery	**109.6%**		**98.0%**		**117.0 %**	

Table 3: Precision Studies of Tepotinib in Standard and sample Dilutions

Inj.No	Name of the drug & Conc (200 µg/ml).	R_t in min. (standard dilutions)	Peak Area (standard dilutions)	Rt in min. (sample dilutions)	Peak Area (sample dilutions)
1	Tepotinib	2.819	7698622	2.812	7661994
2	Tepotinib	2.818	7698577	2.810	7655525
3	Tepotinib	2.817	7693136	2.810	7636160
4	Tepotinib	2.813	7683915	2.810	7624722
5	Tepotinib	2.813	7670573	2.809	7641083
6	Tepotinib	2.812	7668883	2.809	7644532
Mean		2.815	**7685618**	2.810	**7644002.7**
Std. dev.		0.108	13438	0.038	13405
%RSD.		0.003	0.175	0.001	0.175

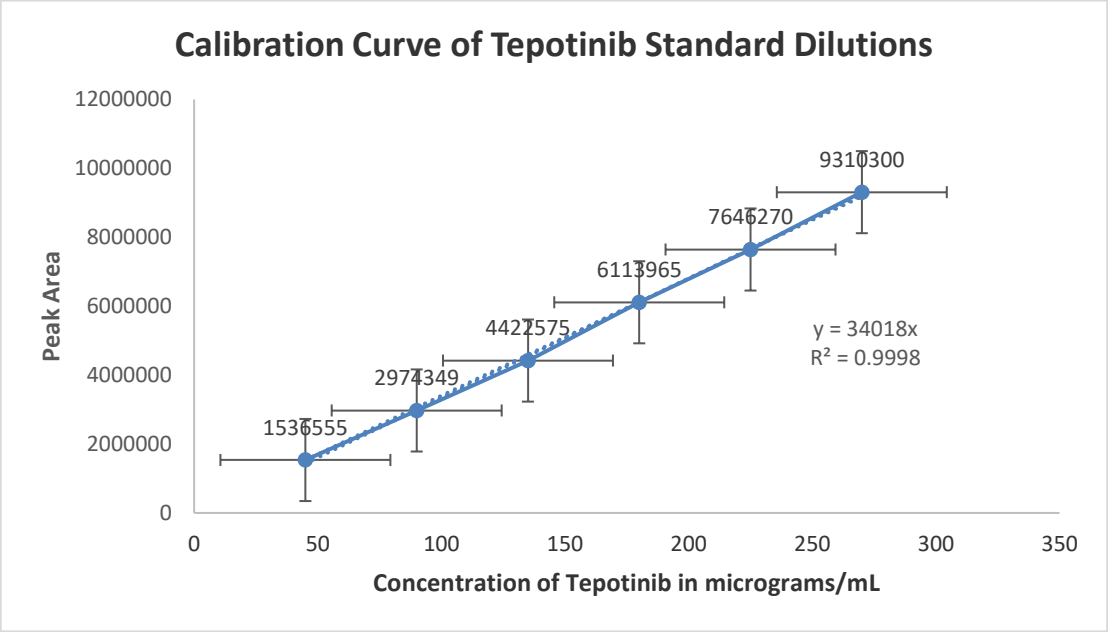

Figure 2: Calibration Curve of Tepotinib standard dilutions:

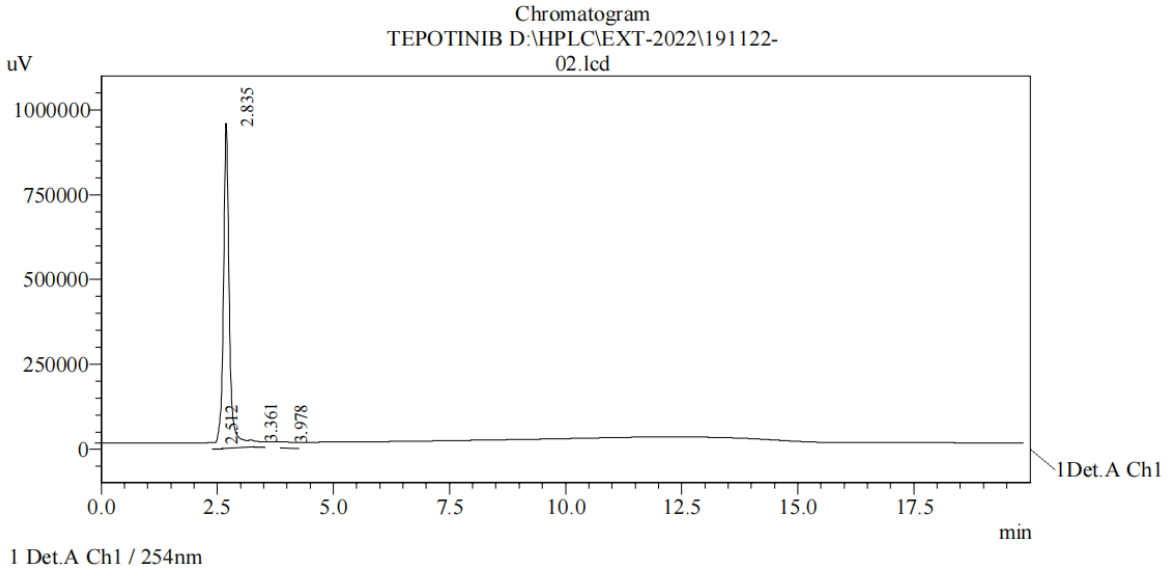

Figure 3: Chromatogram of sample solution

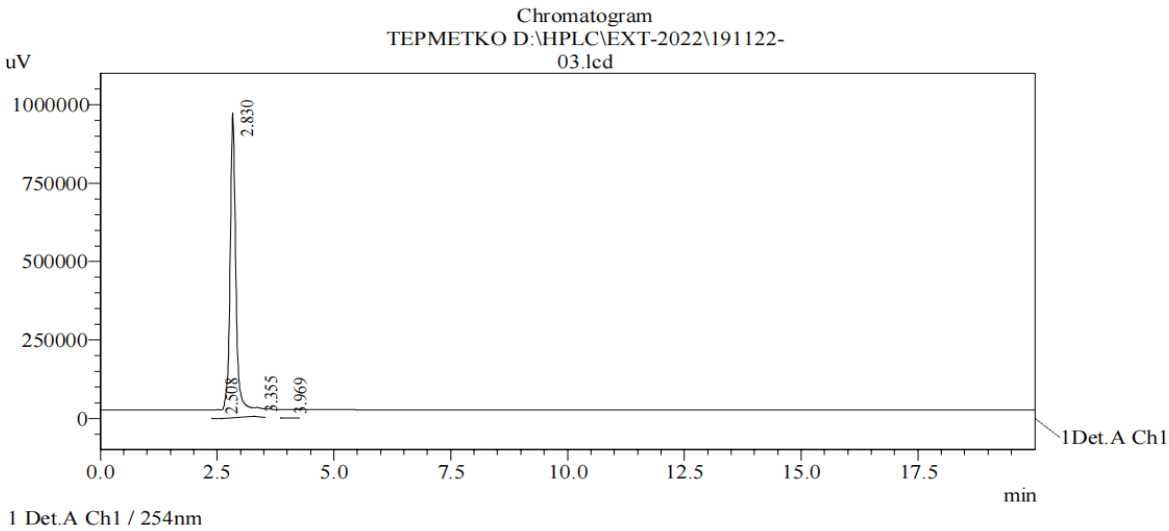

Figure 4: Chromatogram of standard solution

CONCLUSION

In this study, a straightforward HPLC method for the quantification of tepotinib in pharmaceutical matrices was developed. The main benefits of this approach are its simplicity, ease of use, and noticeably shorter run times. In practice, each of these characteristics is crucial, especially when a lot of samples need to be examined. Collectively, the results of the validation tests suggested a method with a respectable linear range, acceptable precision and accuracy, and practically dependable sensitivity. Tepotinib can be analyzed easily, selectively, sensitively, and specifically using this method, which can also be applied to routine analysis in pharmaceutical quality control.

REFERENCES

1. Skoog, D. A., West, D. M., & Holler, F. J, Fundamentals of analytical chemistry,Cengage Learning,2003

2. Snyder, L. R., Kirkland, J. J., & Glajch, J. L,Practical HPLC method development,John Wiley & Sons,2010.

3. Ahuja, S., & Dong, M. W, Handbook of pharmaceutical analysis by HPLC, Elsevier,2010.

4. Swartz, M. E., & Krull, I. S, Analytical method development and validation, CRC Press,2012.

5. Li, W. H., & Li, X. M, Quantitative analysis of pharmaceutical formulations by high performance liquid chromatography, Elsevier, 2013.

6. Kromidas, HPLC made to measure: a practical handbook for optimization, Wiley-VCH,2013.

7. Paik, P.K,Veillon, R.Cortot, A.B. Felip, E.Sakai, H.Mazieres, J.Griesinger, F. Horn, L.Senellart, H. Van Meerbeeck, J.P,Phase II study of tepotinib in nsclc patients with met EX14 mutations, J. Clin. Oncol, 2019;37:9005.

8. Ruiz-Cordero, R. Devine, W.P. Targeted therapy and checkpoint immunotherapy in lung cancer,Surg. Pathol. Clin, 2020;13:17–33.

9. Markham, A. Tepotinib: First approval Drugs 2020; 80: 829–833.

10. Li, F. MacKenzie, K.R.Nyshadham, P. Kerlec, K.A.Matzuk, M.M, Identifying metabolic pathways of c-met tyrosine kinase inhibitor tepotinib in human and mouse liver microsomes, FASEB J. 2020; 34:41-48.

11. Abdelhameed, A.S. Attwa, M.W.Kadi, A.A, Identification of Iminium Intermediates Generation in the Metabolism of Tepotinib Using LC-MS/MS: In Silico and Practical Approaches to Bioactivation Pathway Elucidation, Molecules 2020; 25:5004.

12. ICH guidelines – Q2a and Q2b.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.