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ORIGINAL ARTICLE **OPEN ACCESS**

HPLC Analytical Method Development and Validation for Estimation of Trametinib in API and Pharmaceutical Formulation

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ABSTRACT
A novel, specific, accurate, rugged, precise reversed-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Trametinib in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using Symmetry Xterra C18 (4.6mm x 150mm, 5µm) column with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH-2.8 with orthophosphoric acid in the ratio of 35:65%v/v. The flow rate was 1.0 mL/min and effluent was monitored at 246 nm and a peak eluted at 4.865 min and column oven temperature was maintained ambient. The calibration curve was plotted with a range from 10- 30 µg/mL. The LOD and LOQ values of Trametinib were found to be 1.3µg/mL and 3.9µg/mL respectively. The percentage recovery of Trametinib was found to be within the limits. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision, and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, and accurate, which is useful for the routine determination of Trametinib in bulk drug and its pharmaceutical dosage form. The proposed method was applied for the analysis of tablet formulations, to improve QC and assure therapeutic efficacy.
Keywords*: Trametinib, RP-HPLC, Accuracy, Precision, Validation, ICH Guidelines.*

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INTRODUCTION

High-performance liquid chromatography (HPLC) is a technique used for the analysis of drug substance, drug product and determination and quantification of known as well as unknown impurities at the lower level, food and drug administration (FDA) also trust the purity method of analysis by using HPLC, because of high accuracy and reproducibility of results. By using this technique we can separate drug-related process impurities, degradation impurities as well as reactants [1].

According to the principle of separation of HPLC, as the particle size of column material decreases, the efficiency of the chromatographic separation, speed, and resolution also increases. The HPLC is the most simple, economic, reliable, and worldwide used technique in pharmaceutical analysis [2].

Cancer patients are facing a number of problems due to the disease itself or even with its treatment. Clinical anxiety and depression are apparent in one-third of cancer patients [3]. Prolongation of survival, palliation of symptoms, and preservation of quality of life are the main goals of chemotherapy. Even though chemotherapy can lead to nausea, vomiting, alopecia, fatigue, sexual dysfunction, and reduction in quality of life [4]. The prevalence of cancer pain is estimated at 25% for those newly diagnosed, 33% for those undergoing active treatment, and greater than 75% for those with advanced disease. Pain prevalence is also high in specific cancer types such as head and neck cancer (49%). With such a high prevalence, cancer pain should be anticipated and responded to as early as possible [5, 6].

Trametinib is chemically N-(3-{3-Cyclopropyl-5-[(2- fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo3,4,6,7-tetrahydropyrido[4,3-d] pyrimidine-1(2H)- yl} phenyl) acetamide. Trametinib (trade name Mekinist) is a cancer drug. It is a MEK inhibitor drug with anticancer activity[7]. It inhibits MEK1 and MEK2. Trametinib had good results for metastatic melanoma carrying the BRAF V600E mutation in a phase III clinical trial [7].

From the literature survey, it was revealed that few chromatographic analytical methods have been developed for the determination of Trametinib in pharmaceutical preparations [7, 8]. Therefore, the current work aimed to develop an accurate, specific, and robust RP-HPLC technique for the assessment of Trametinib, as a single drug under the International Conference on Harmonization (ICH) guidelines.

MATERIAL AND METHODS

Trametinib (Pure) from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Potassium Dihydrogen Phosphate from Finar Chemicals.

Optimization of Column

The method was performed with various C18 columns like Symmetry, Zodiac, and Xterra. Symmetry Xterra C18 (4.6mm x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1mL/min flow.

Optimized Chromatographic Conditions

An instrument used: Waters HPLC with autosampler and PDA 996 detector model.
Temperature: Ambient
Column: Symmetry Xterra C18 (4.6mm x 150mm, 5µm)
Mobile phase: Acetonitrile: Phosphate Buffer (Ph-2.8) (35:65% v/v)
Flow rate: 1.0 mL/min
Wavelength: 246 nm
Injection volume: 10 µl
Run time: 8 minutes

Preparation of mobile phase

Accurately measured 350 mL of Acetonitrile (35%) and 650 mL (65%) Phosphate Buffer were mixed and degassed in a digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Method validation

Method validation was executed by ICH recommendations for system suitability, accuracy, specificity, linearity, precision, sensitivity, and robustness [9, 10].

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value [11].

$$LOD = 3.3 \times \sigma / s$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Limit Of Quantitation (LOQ)

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined[11].

$$LOQ = 10 \times \sigma / S$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

RESULTS AND DISCUSSION

System suitability

In System suitability, injecting standard solution and reported USP tailing and plate count values are tabulated in Table-1. The tailing factor was found to be 1.26 for Trametinib, which represents the peak was symmetric. According to chromatographic peak results, the developed analytical method satisfies the criteria for system suitability, and the sample chromatogram was shown in Fig-1.

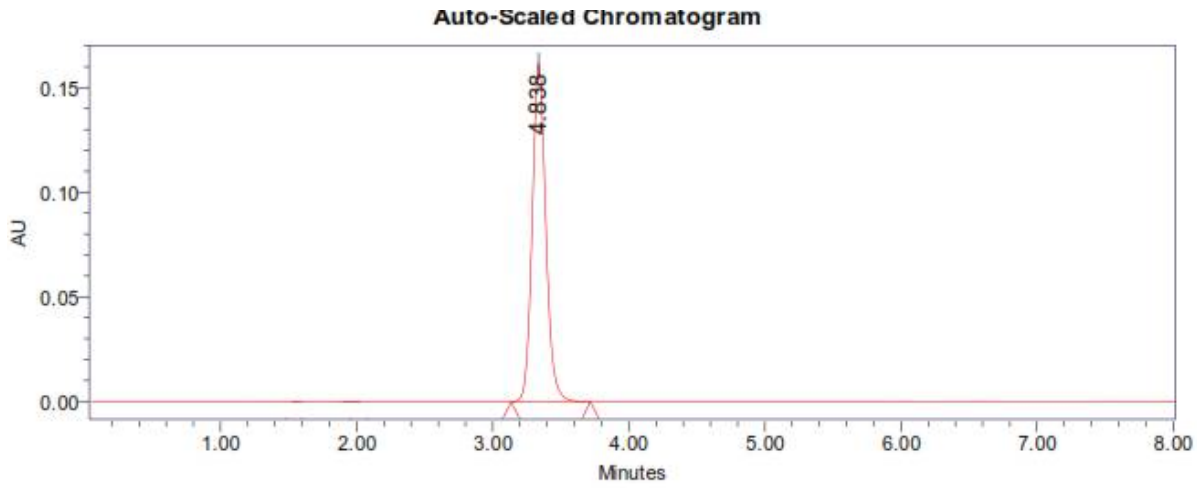

Fig-1: Chromatogram showing system suitability injection

Table-1: Results of system suitability for Trametinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Trametinib	4.865	856985	69854	8569	1.26
2	Trametinib	4.838	856857	68954	8547	1.25
3	Trametinib	4.846	857894	68975	8596	1.25
4	Trametinib	4.844	857468	69854	8541	1.26
5	Trametinib	4.838	854785	69856	8616	1.25
Mean			856797.8			
Std. Dev.			1197.992			
% RSD			0.139822			

Assay: The % purity of Trametinib in the pharmaceutical dosage form was found to be 99.87%. The percent assay value is near 100 percent. In Table-2, 3; we can see the results of Peak Area, USP Plate Count, and USP Tailing.

Table-2: Results of Assay (Standard) for Trametinib

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Trametinib	4.838	866854	70152	8659	1.26
2	Trametinib	4.840	868478	69987	8657	1.27
3	Trametinib	4.838	865987	70154	8654	1.26
4	Trametinib	4.842	865896	69985	8659	1.27
5	Trametinib	4.846	859864	69587	8674	1.27
Mean			865415.8			
Std. Dev.			3272.034			
% RSD			0.378088			

Table-3: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trametinib	4.840	875845	70025	1.28	8659	1
2	Trametinib	4.842	876584	70066	1.27	8696	2
3	Trametinib	4.834	874598	69989	1.28	8785	3

LINEARITY: Linearity Regression equation for Trametinib was found to be y = 42159 x+11463. The linear concentration range was found to be 10-30 µg/mL with a value of 0.999 for the regression coefficient for the Trametinib compound. The calibration curve for Trametinib is given in Fig-2.

Table-4: Data for Linearity of Trametinib

Concentration µg/mL	Average Peak Area
10	442986
15	652547
20	856985
25	1063654
30	1268475

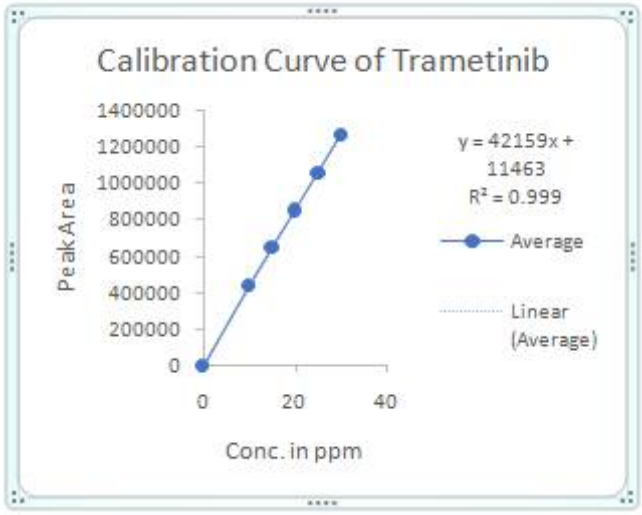

Fig-2: Calibration Curve of Trametinib

Precision: The precision of the analytical technique is the degree of proximity of the sequence of measurements obtained from multiple homogeneous samplings[12]. The accuracy of the process of the drug was calculated by injection of six individual determinations of Trametinib (20 µg/mL). Method precision results were shown in Table-5.

Table-5: Results of method precision for Trametinib

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Trametinib	4.840	856985	69856	8569	1.26
2	Trametinib	4.842	856898	69845	8597	1.25
3	Trametinib	4.834	856789	69865	8589	1.26
4	Trametinib	4.838	859854	69874	8569	1.25
5	Trametinib	4.846	854789	69798	8564	1.26
6	Trametinib	4.844	856978	69859	8599	1.25
Mean			857048.8			
Std.dev			1617.106			
%RSD			0.188683			

Ruggedness: The ruggedness of the method is determined by the comparison of the results of the assay from two different laboratories and two analysts. The % RSD values on the assay of Trametinib from two different laboratories by two analysts were not more than 2%, which indicates the ruggedness of the developed method [11-12].

Table-6: Results of ruggedness for Trametinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trametinib	4.882	865845	70023	8659	1.27
2	Trametinib	4.877	864356	70015	8667	1.27
3	Trametinib	4.877	867584	69989	8654	1.28
4	Trametinib	4.882	865987	70114	8645	1.28
5	Trametinib	4.877	865975	69985	8635	1.27
6	Trametinib	4.877	865982	69998	8695	1.28
Mean			865954.8			
Std. Dev.			1022.223			
% RSD			0.118046			

Accuracy: The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent, and 150 percent). APIs with concentrations of 10, 20, and 30 µg/mL of Trametinib were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test process. The recovery results were similar to 100%. The percentage recovery, mean and relative standard deviations were determined [13]. Recovery values shown within the desired range were correct. The results are summarized below. Accuracy findings have been shown in Table-7.

Table-7: The accuracy results for Trametinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	429549.7	10	9.916	99.16%	99.68%
100%	856189.3	20	20.036	100.18%	
150%	1272534	30	29.912	99.706%	

Limit of detection and quantification for Trametinib

Standard error and slope of linear data are used to predict the LOD and LOQ of Trametinib and precision was established at the predicted concentration. The result was shown in the Table.

Table-8: Limit of detection and Limit of quantification

Limit of detection	Limit of quantification
1.3µg/mL	3.9µg/mL

ROBUSTNESS: The flow rate was altered by ± 0.1 mL/min, and the acetonitrile proportion in the mobile phase was altered by ± 5%[13]. The observed % RSD was found less than 2%, There was not much difference in peak Area and retention time justifying the method is robust and suitable for the estimation of Trametinib (Table-9).

Table-9: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
The actual Flow rate of 1.0 mL/min	856985	4.865	8547	1.25
Less Flow rate of 0.9 mL/min	841542	5.021	8256	1.23
More Flow rate of 1.1 mL/min	812546	3.997	8146	1.20
Less organic phase	802654	6.242	8365	1.16
More organic phase	826549	4.002	8154	1.14

CONCLUSION

This method described the quantification of Trametinib in bulk and pharmaceutical formulation as per ICH guidelines. The evolved technique was found to be rapid, accurate, precise, linear, robust, economical, and reliable. The advantage lies in the simplicity of sample preparation and the reproducibility of data is satisfactory. The evolved chromatographic method can be effectively applied for regular investigation in drug research. The mobile phase is simple to prepare and economical. This method is also having the advantage that the retention time of the drug is below 5 min and the drug can be assayed within a short time. Thus, the method is not time-consuming and can be used in laboratories for the routine analysis of single and combination drugs.

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