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Determination of trametinib in pharmaceutical formulations by rp-hplc method

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ABSTRACT

A new simple, accurate, economic, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Trametinib in bulk form and its pharmaceutical dosage form. Chromatographic separation was carried out on Zorbax C18 (4.6mm x 250mm, 5µm, Make: X terra) column using a mixture of Acetonitrile: Methanol: Water (50:30:20% v/v) as the mobile phase at a flow rate of 1.0 ml/min, the detection was carried out at 245nm. The retention time of the Trametinib was found to be 5.462 ±0.02min. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 50-90 mcg/mL for Trametinib. The correlation coefficient was found to be 0.999. The LOD and LOQ for Trametinib were found to be 1.6µg/mL and 4.8µg/mL respectively. The proposed method was found to be good percentage recovery for Trametinib, which indicates that the proposed method is highly accurate. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence the developed method was successfully applied to determine Trametinib in pharmaceutical formulations

Keywords: Trametinib, RP-HPLC, Method Development, Validation, ICH Guidelines.

INTRODUCTION

Trametinib (TRB) 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 anti cancer drug.(Fig.1) It is a MEK inhibitor drug with anticancer activity[1]. It inhibits MEK1 and MEK2. Trametinib had good results for metastatic melanoma carrying the BRAF V600E mutation in a phase III clinical trial. In this mutation, the amino acid valine (V) at position 600 within the BRAF protein has become replaced by glutamic acid (E) making the mutant BRAF protein constitutively active.]Clinical trial data demonstrated that resistance to single-agent Trametinib often occurs within 6 to 7 months.[2-5] To overcome this, Trametinib was combined with the BRAF inhibitor dabrafenib. As a result of this research, on January 8, 2014, the FDA approved the combination of dabrafenib and Trametinib for the treatment of patients with BRAF V600E/K-mutant metastatic melanoma.[5-8] Trametinib is used alone or in combination with dabrafenib (Tafinlar) to treat a certain types of melanoma (a type of skin cancer) that cannot be treated with surgery or that has spread to other parts of the body.

Literature review revealed that only pharmacological and clinical studies [8-13] have been

reported for the determination of Trametinib and a pharmacokinetic study which used LC-MS method for the determination of entacapone[9]. HPLC has become a widely used tool for the routine determination and separation of drugs either alone in pure form or in admixture with other drugs or degradation products and in pharmaceutical formulations.[10-13]Existing literature reveals that there are only few methods for the assay of Trametinib in bulk and dosage forms [14].

There is no official method for the estimation of Trametinib in the pharmacopoeia`s. Hence an attempt has been made to develop a new simple, reliable, and reproducible, isocratic RP-HPLC methods to estimate the Trametinib in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility respectively. The proposed method was validated as per ICH guidelines [14-21].

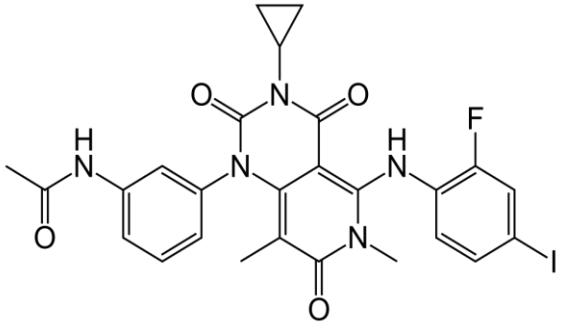

Figure.1 :*Structure of* Trametinib

Experimentation

Equipment

Chromatographic separation was performed on Waters HPLC with auto sampler and PDA detector 996 model consist of PDA detector and auto sampling unit. Empower software was applied for data collecting and processing.

Reagents and chemicals

Acetonitrile, Methanol and water of HPLC grade were procured from Rankem lab ltd. Trametinib was received as gift samples from Hetero Labs Ltd., Hyderabad, India, respectively. MEKINIST® tablets were purchased from local market.

HPLC conditions

A Zorbax C18 (4.6mm x 250mm, 5µm, Make: X terra) column was used as the stationary phase. A mixture of Acetonitrile: Methanol: Water (50:30:20% v/v), in the ratio of 50:30:20 was used as a mobile phase. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 245 nm.The injection volumes of samples and standard were 20µl.

Standard solutions

A stock solution containing 1000µg/ml of TRB was prepared by dissolving TRB in mobile phase. A working standard solution containing 50-90µg/ml of TRB was prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

Assay of tablet formulation

20 tablets (each tablet contains 2 mg of Trametinib (MEKINIST ®) flim coated tablets were accurately weighed and calculated their average weight. Then it was taken into a mortar and crushed to fine powder and uniformly mixed. A quantity of powder equivalent to10 mg of TRB was weighed and transferred to a 10 ml standard flask .The drug was initially dissolved in diluent and sonicated for 10 minutes. The volume was made up to 10 ml with mobile phase. Then the solution was filtered using 0.45-micron syringe filter. After that 0.7 ml of the above filtrate was diluted to 10 ml with the diluent so as to give a concentration of 70µg/ml of Trametinib .Then 20µl of this solution was injected in to the column and chromatogram was recorded and shown in Fig.2.Each concentrations of TRB in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in table1.

Table 1: Table for Assay

Tablet formulation	Drug	Amount present (mg/tab)	Amount found* (mg/tab)	% label claim*
T1	TRB	2	1.99	99.91 %
T2	TRB	5	4.98	99.98%

T1 and T2 are two different brands of tablet formulations. ENT denotes Trametinib respectively.*Each value is average of six determinations.

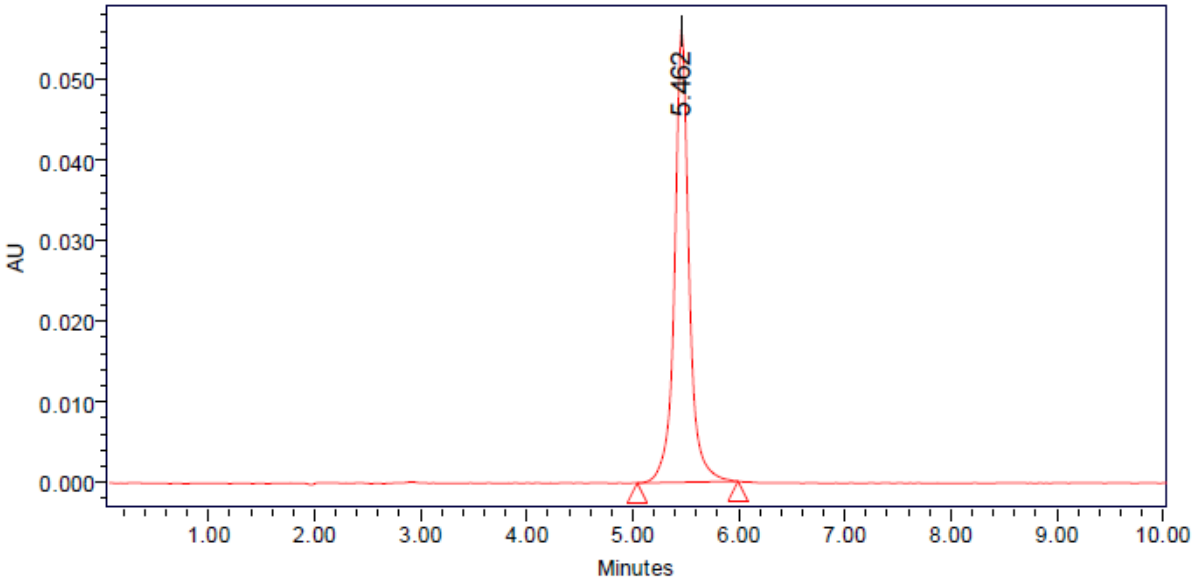

Figure 2 : Assay Chromatogram of Trametinib

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.7ml of the above Trametinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and Methanol: Phosphate buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Methanol: Water (50:30:20% v/v) respectively.

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6mm x 150mm, 5µm, Make: X terra) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow. (Figure No3)

Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Mobile phase ratio : Acetonitrile: Methanol: Water (50:30:20% v/v)
Column : Zorbax C18 (4.6mm x 250mm, 5µm, Make: X terra)
Column temperature : 35^0C
Wavelength : 245 nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 10min

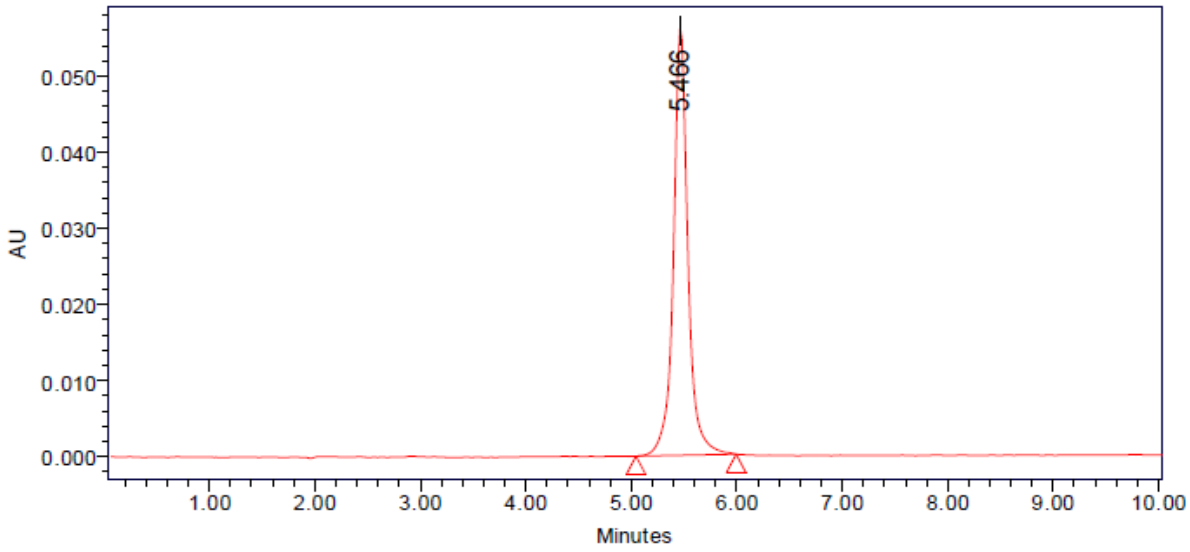

Figure 3: Optimized Chromatographic Condition

Validation of the method

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 500 ml (50%) of Acetonitrile, 300 ml (30%) of Methanol and 200 ml of HPLC Grade water (20%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µm filter under vacuum filtration.

Table 2: System suitability results of Trametinib

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	5.474	1052658	9658	1.43
2	5.466	1058475	9758	1.42
3	5.474	1059854	9759	1.44
4	5.452	1054786	9747	1.45
5	5.446	1052642	9797	1.44
6	5.421	10555194.1	9795	1.48
Mean	5.455	6549	-	-
SD	0.018	2931.25	-	-
%RSD	0.20	0.497	-	-

Diluent Preparation

The Mobile phase was used as the diluent.

System suitability studies

The system suitability test was carried out on freshly prepared stock solution of Trametinib to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in table 2. The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within ± 3% standard deviation range during routine performance of the method.

Linearity and Range

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 50-90 µg/ml. 20µl of

each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas (Y axis) against the amount of drug in µg/ml(X axis). Peak area of linearity range and the parameters were calculated and presented in table 3 respectively. The linearity curve of Trametinib was shown in Figure.4.

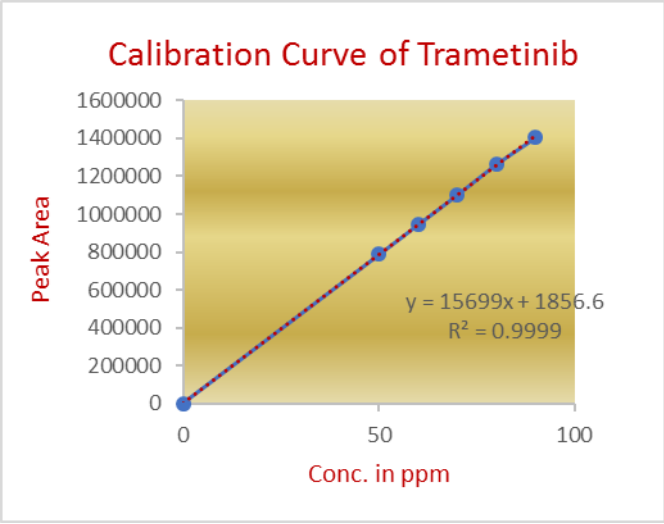

Figure.4: Linearity curve of Trametinib

Table 3: Analytical performance parameters of linearity curve

S.No	parameters	Trametinib
1	Linear dynamic range(µg/ml)	50-90
2	Correlation coefficient	0.9999
3	Slope (m)	15699
4	Intercept	1856.6
5	Curve fitting	99.99
6	LOD(µg/ml)	1.6
7	LOQ(µg/ml)	4.8

Limit of detection and Limit of quantification

The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD= 3.3X {Residual Standard deviation/Slope}.

The LOD for Trametinib was confirmed to be 0.5 µg/ml.

The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ The LOQ for Trametinib was confirmed to be1.5 µg/ml

Accuracy

The accuracy of the method was determined by recovery experiments. Placebo was spiked with known quantities of standard drugs at levels of 80 to 120% of label claim. The recovery studies were carried out 3 times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in table 4. The mean recovery is well within the acceptance limit, hence the method is accurate.

= 10X {Residual Standard deviation/Slope}

Table 4: Recovery studies of Trametinib

Labeled	Amount mg/tab Found*	%Label Claim (n=6)	Recovery Studies (n=3)			
			Amount added(mg)	Amount recovered (mg)	%Recovery	% RSD
Trametinib 5 mg	2.103	100.25±0.2971	4	4.11±0.125	100.921	0.274
			5	5.04±0.23	100.24	0.525
			6	5.97±0.02	99.14	0.541

Average of six or three determinations, Mean ± Standard Deviation

Precision

System precision

The system precision of the method was established by six replicate injections of the standard solution containing Trametinib. The percentage RSD were calculated and presented in Table 5. From the data obtained, the developed RP-HPLC method was found to be precise.

Method precision

The method precision of the method was established by carrying out the analysis of Trametinib (n=6) using the proposed method. The low value of the relative standard deviation showed that the method was precise. The results obtained were presented in table 5.

Table 5:Precision studies of Trametinib in dosage forms

	System Precision		Method Precision	
	Trametinib		Trametinib	
S.No.	**Retention time**	**Peak Area**	**Retention time**	**Peak Area**
1	5.419	1052658	5.484	1075846
2	5.405	1056854	5.493	1078254
3	5.478	1052468	5.406	1078598
4	5.466	1052774	5.419	1075461
5	5.466	1055245	5.446	1075236
6	5.661	1055125	5.452	1075842
Avg	5.482	1054187	5.451	1075842
Stdev	0.092	1811.15	0.0344	1483.68
%RSD	**1.64**	**0.171**	**0.555**	**0.137**

Specificity

Specificity of the method was determined by injecting the diluted placebo. There was no interference of placebo with the principle peak, hence the developed analytical method was specific for Trametinib in tablet dosage form.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature and refrigerator temperature for 24h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were up to 24h at room temperature and refrigerator temperature.

Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010 A4T), Water Alliance HPLC 2695 by different operators using different columns of similar type like Hypersil C_{18} column and Xterra C18 column. Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The results of ruggedness were presented in table 6. The results of robustness were presented in table 7.

Table 6: Method ruggedness of Trametinib in dosage forms

%Assay*(n=6)	**%RSD ofAssay(n=6)**
Day -1 , Analyst-1, Instrument-1&Column-1	
100.91 ± 0.112	0.523
Day -2 , Analyst-2, Instrument-2&Column-2	
100.29 ± 0.321	0.121

**Average of six determinations, mean ± Standard Deviation*

Table 7: Method robustness of Trametinib in dosage Forms

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1052689	5.453	9625	1.62
Less Flow rate of 0.9 mL/min	1015241	5.599	9155	1.54
More Flow rate of 1.1 mL/min	1023654	4.576	9254	1.56
More Organic phase	1015853	3.827	9147	1.54
Less organic phase	1002514	7.415	9256	1.53

CONCLUSION

The proposed RP-HPLC method for the estimation of Trametinib in tablet dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in determination of Trametinib in bulk form and pharmaceutical dosage form.Hence the present RP-HPLC method is suitable for the quality control of the raw material, formulation and dissolution studies.The method validation shows satisfactory data for all the method validation parameter tested.

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