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Analytical method development and validation for the quantitative estimation of trametinib in api and marketed tablet dosage form by using hplc method

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ABSTRACT

A Correct, particular, easy and reproducible, isocratic Reversed section high overall performance Liquid chromatography (RP-HPLC) Technique became developed and established for the simultaneous estimation of trametinib in API and marketed dosage form. Trametinib were separated through the usage of a symmetry C18 ODS (4.6mm 250mm) 5m particle size HPLC WATERS with Empower to software with Isocratic by using UV- Visible Detector. Mobile phase consist of Acetonitrile: phosphate buffer (0.01M,pH-3.2 (30:70v/v). The flow rate has become set to 1ml/min with responses measured at 246 nm. The retention time was 5.453 min. Linearity emerge with correlation coefficient is 0.999.The present method was validated as per guidelines of International conference on Harmonization (ICH) including parameters like specificity, linearity, precision, accuracy, and robustness, restriction of detection (LOD) and restrict of quantitation.

Keywords: Methanol, UV spectrophotometric estimation, RP-HPLC Method development, Validation.

INTRODUCTION

Trametinib is an Anti-Neoplastic Agent, and it is a reversible, allosteric inhibitor of mitogen-activated extracellular signal regulated kinase 1 (MEK1) and MEK2 activation and of_ MEK1_ and MEK2 kinase activity. MEK proteins are upstream regulators of the extracellular signal-related kinase (ERK) pathway, which promotes cellular proliferation. Trametinib helps with melanoma with the BRAF V600E or V600K as the mutation results in the constitutive activation of the BRAF pathway which includes MEK1 and MEK2. Trametinib is an orally bioavailable inhibitor of mitogen-activated protein kinase (MAP2K; MAPK/ERK kinase; MEK) 1 and 2, with potential antineoplastic activity. Upon oral administration, Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in an inhibition of growth factor-mediated cell signaling and cellular proliferation in various cancers. MEK 1 and 2, dual specificity serine/threonine and tyrosine kinases often upregulated in various cancer cell types, play a key role in the activation of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth.

According to literature review here are very few methods reported for the determination of Trametinib in different Instrumental techniques, out of these methods R P - H P L C methods were also few.

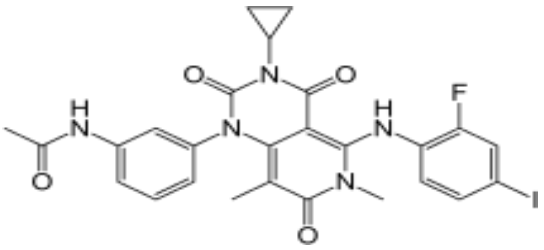

Fig 1: Structure of Trametinib

IUPAC Name: N-[3-[3-cyclopropyl-5-(2-fluoro-4-iodoanilino)-6,8-dimethyl-2,4,7- tri oxo pyrido[4,3-d] pyrimidin-1-yl] phenyl] acetamide
Molecular Formula: C26H23FIN5O4
Molecular Weight: 615.3948g/mol
Physical Appearance: White to white odorless powder.
Solubility: soluble in organic solvents such as DMSO and dimethyl formamide.

EXPERIMENTAL SECTION

Standard drugs

2mg Mekinist GlaxoSmithKline

Chemicals and reagents

HPLC grade water, Methanol HPLC Loba Chem, Ethanol A.R. Sd fine-Chem ltd, Acetonitrile HPLC Loba Chem, DMSO A.R. Sd fine-Chem ltd, DMF A.R. Sd fine-Chem ltd.

Instruments

HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2. T60-LABINDIA UV – Vis spectrophotometer
3. High Precision Electronic Balance
4. Ultra Sonicator (Wensar wuc-2L)
5. Thermal Oven
6. Symmetry C18 Column, 250 mm x 4.6 mm and 5µm particle size
7. P H Analyser (ELICO)
8. Vaccum Filtration Kit (Labindia

Determination of absorption maxima by UV/Visible Spectrophotometry

The Standard Stock Solutions – 10 mg of Trametinib standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Trametinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Trametinib.

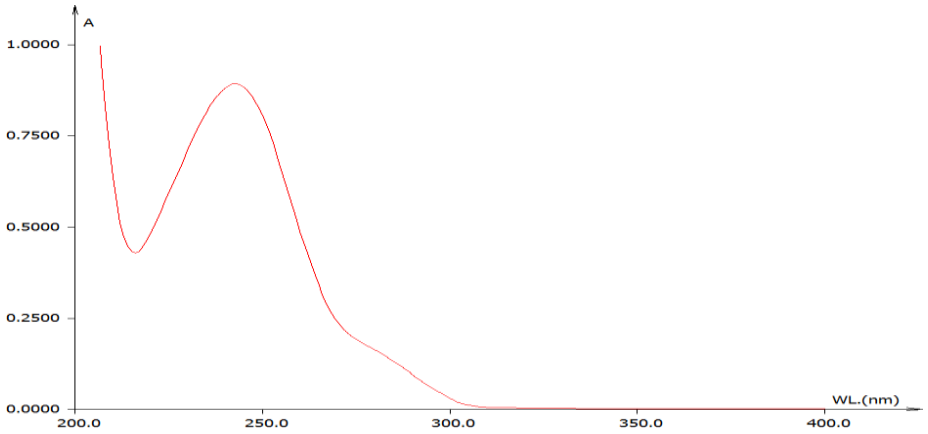

Fig 2: UV spectrum of Trametinib

Estimation of Trametinib in bulk and tablet dosage form

Preparation of mobile phase

Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.
Preparation of 0.01M Potassium dihydrogen orthophosphate Buffer Solution: About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid

Diluent

Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Standard preparation

Accurately weigh and transfer 10 mg of Trametinib, working

standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent. Further pipette 0.1ml of Trametinib from stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation

Take average weight of Tablet and crush in a mortar by using pestle and taken weight 10 mg equivalent weight of Trametinib sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.1ml of Trametinib from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Optimized chromatographic conditions

Mobile phase : Acetonitrile: Phosphate buffer (0.01M, pH-3.2) (30:70v/v) Column : Symmetry C18 ODS (4.6mm×250mm) 5µm particle size Flow rate : 1 ml/min Wavelength : 246 nm Column temp : Ambient Injection Volume : 20 µl Run time : 10 minute

Method validation

The following parameters were considered for the analytical method validation of Trametinib in bulk form & tablet dosage form.

System Suitability

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor time and theoretical plates.

Accuracy

For preparation of 50% Standard stock solution:
Further pipette 0.05ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
For preparation of 100% Standard stock solution:
Further pipette 0.1ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
For preparation of 150% Standard stock solution:
Further pipette 0.15 ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Trametinib and calculate the individual recovery and mean recovery values. The %RSD for each level should not be more than 2.

Precision

Repeatability

Preparation of Trametinib for Precision

Further pipette 0.1 ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Linearity and range

Preparation of Level – I (6µg/ml of Trametinib): Further pipette 0.06 ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – II (8µg/ml of Trametinib): Further pipette 0.08 ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – III (10µg/ml of Trametinib): Further pipette 0.1ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – IV (12µg/ml of Trametinib): Further pipette 0.12ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of Level – V (14µg/ml of Trametinib): Further pipette 0.14ml of Trametinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. Correlation coefficient should be not less than 0.999.

Limit of Detection

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

Limit of Quantitation

The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of Variation of flow Rate

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 70:30, 75:25 instead of 65:35, remaining conditions are same. 20µl of the above sample was injected and chromatograms.

RESULTS AND DISCUSSION

Standard preparation

Preparation of 0.01M Potassium dihydrogen orthophosphate Buffer Solution

About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid.

10 mg of Trametinib working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Trametinib working standard solution. The solution was mixed well and filtered through 0.45µm filter.

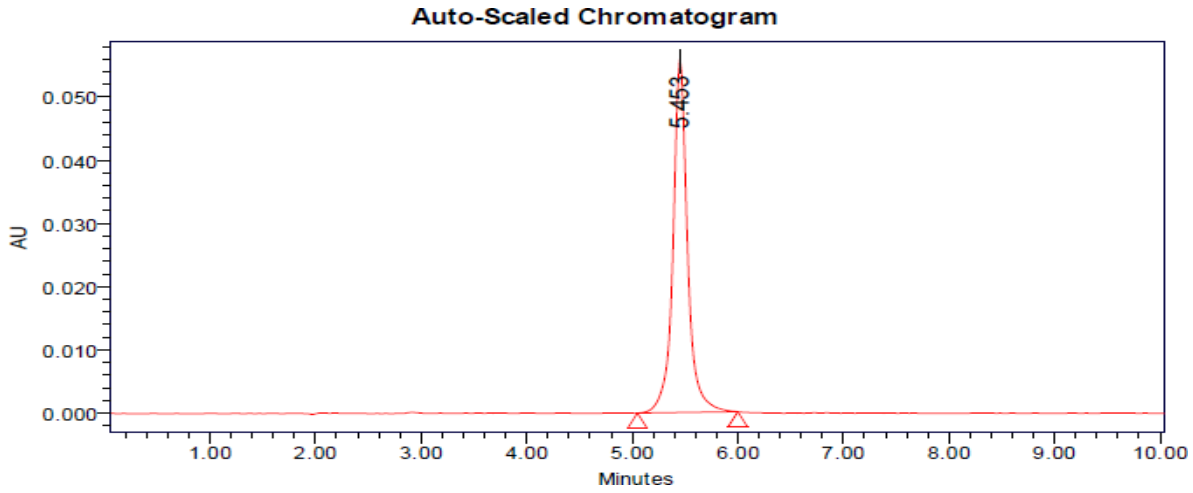

Fig 3: Shows Chromatogram of Trametinib standard

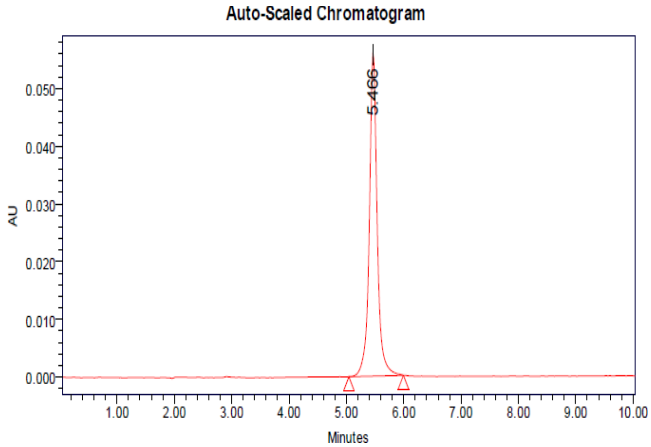

Fig 3.1: Chromatogram of Trametinib sample

Accuracy

Average recoveries of Trametinib are 100.00%, 99.650%, 100.750%, at 50%,100% & 150% concentrations level respectively. The percentage recoveries of the drug is

within the limits 98-102%. So the method is accurate, accuracy data for Trametinib are presented below, in Table No: 1.

Precision

Precision are summarized in Table 2, respectively. The % RSD values for Precision was less than 2.0%, which indicates that the proposed method is precise. The accuracy for the average of triplicate in each concentration samples are within the limit.

Table 1: Shows Accuracy Results of Trametinib

Concentration level	Amount added (ppm)	Amount found(ppm)	%Recovery	Mean recovery
50%	5	5.00	100.000%	
100%	10	9.965	99.650%	100.130%
150%	15	15.111	100.750%	

Table 2: Shows Precision Results of Trametinib

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1.	Trametinib	5.419	645784	83685	6825	1.05
2.	Trametinib	5.405	642589	84932	6849	1.09
3.	Trametinib	5.478	643658	85847	6845	1.08
4.	Trametinib	5.466	648759	86259	6845	1.09
5.	Trametinib	5.493	649657	86587	6895	1.07
6.	Trametinib	5.466	647854	87853	6874	1.10
Mean			646383.5			
Std. Dev			2853.319			
%RSD			0.441428			

Linearity

The response was found linear over concentration range of 6-14 µg/mL of Trametinib. The correlation co-efficient were found to be 0.999 for Trametinib, So the method is linear, data is presented in Table: 4.

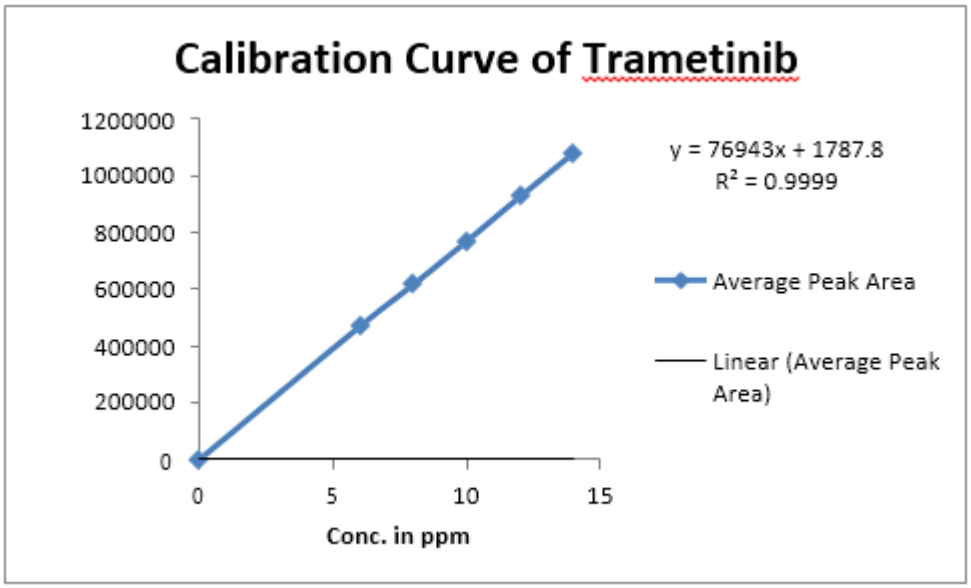

Table 4: Linearity results of Trametinib

S.no	Linearty level	Concentration	Area
1	I	6µg/ml	468784
2	II	8	615798
3	III	10	768759

4	IV	12	925748
5	V	14	1078765
Correlation Coefficient			0.999
Intercept Slope			Y=76943x+1787.876943

Robustness

The Robustness of the method was determined by making slight changes in the experimental conditions such as change in the Flow rate, & phase changes.

Table 5: Results of Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	648759	5.484	6845	1.08
Less Flow rate of 0.9 mL/min	635248	5.599	6786	1.09
More Flow rate of 1.1 mL/min	659865	4.576	6528	1.05
Less organic phase	625986	7.415	6689	1.03
More organic phase	615869	3.827	6354	1.01

Limit of Detection (LOD) & LOQ

The detection limit is determined by the analysis of samples with known concentration of analyse and by establishing that minimum level at which the analyse can reliably detected**,** The LOD are calculated from the calibration curve by formula LOD = 3.3 x SD/ s The quantification limit is generally determined by the analysis of sample with known concentrations of analyse and by establishing the minimum level at which the analyse can be quantified with acceptable accuracy and precision, The LOQ are calculated from the calibration curve by formula LOQ = 10 x SD/ s.

Table 6: Summary of validation parameter Results

S.NO	Parameter	Acceptance criteria	UV
1	%Recovery	98-102%	100.130%
2	Linearity range (µg/ml)	-	6-14(µg/ml)
3	Correlation Coefficent	NLT 0.999	0.999
4	Precision	%RSD(NMT 2%)	0.441428
5	Intermediate Precision	%RSD(NMT 2%)	0.258918 & 0.373433
6	Robustness	%RSD(NMT 2%)	1.0911
7	LOD		0.487µg/ml
8	LOQ		1.477µg/ml

CONCLUSION

A sensitive and particular RP-HPLC technique has been created and approved for the examination of Trametinib in API form and Marketed Tablet Dosage form. Promote the proposed RP-HPLC technique has magnificent affectability, exactness and reproducible. In future the developed method for the Trametinib in API and Marketed Tablet Dosage Form can be utilized by Industry and further the extension of research can be done and this simple method can also utilized for studying by the students in the universities.

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