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Method development and validation for the quantitative estimation of Trametinib in API form and marketed tablet dosage form by RP-HPLC

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Abstract
The development of analytical methods is in need for the estimation of Trametinib in pure and different pharmaceutical formulations. A simple, sensitive, rapid, accurate, precise and economic chromatographic method was developed and validated for Trametinib in pure and pharmaceutical formulations. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines. This separation is performed on Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm Column and mobile phase consists of ACN : Methanol: 0.1% OPA in the ratio of 60:30:10v/v/v at flow rate of 1.0ml/min. The wave length (λmax) used for the estimation of Trametinib is 267 nm by chromatographic method. The linearity of the calibration curve was validated by the high values of the correlation coefficient of regression. The percentage of Trametinib recovered was found to be within the limits i.e. 98-102% for Trametinib. LOD and LOQ values for Trametinib were found to be 0.08µg/ml and 0.24µg/ml respectively. The developed methods are simple and suitable for the determination of Trametinib in pure and pharmaceutical preparations.

Keywords: Trametinib, RP-HPLC, ICH Guidelines, Robustness, Linearity, Precision

Introduction

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 [1] 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. Trametinib is an anticancer agent which causes apoptosis (or programmed cell death) and inhibits cell proliferation, which are both important in the treatment of malignancies. Trametinib [2] 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 indicated for the treatment of unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test [FDA]. Trametinib [3] 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. The IUPAC Name of Trametinib is N-[3-[3-cyclo propyl-5-(2-fluoro-4-iodo anilino)-6, 8-dimethyl-2, 4, 7-trioxo pyrido [4, 3-d] pyrimidin-1-yl] phenyl] acetamide. The Chemical Structure of Trametinib is as following growth opportunities, according to several scholars (Chew *et al*., 2013).

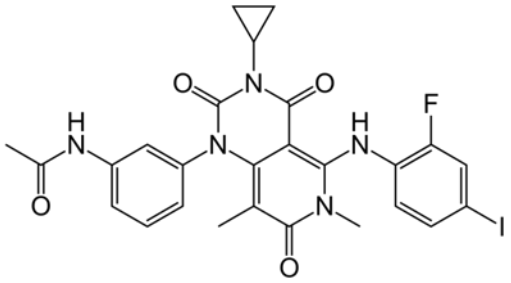

Fig 1: Chemical Structure of Trametinib

Materials and Methods

Instruments used

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Chemicals / Reagents Used

Table 2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	99.9%	A.R.	Loba Chem; Mumbai.
8.	Hydrogen Peroxide	99.9%	A.R.	Loba Chem; Mumbai.

Method Development and its Validation for Trametinib by RP-HPLC

Method Development

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. 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. The scanned UV spectrum is attached in the following page,

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Trametinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution [4] was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

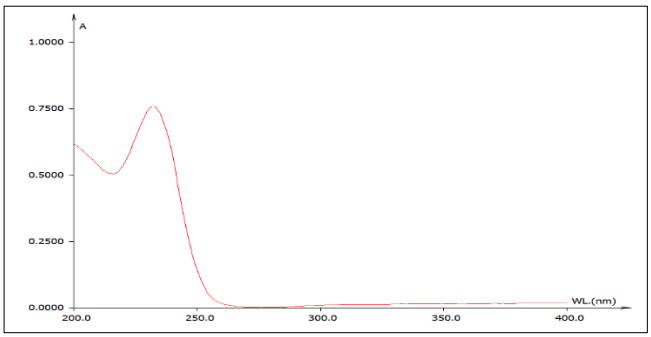

Fig 2: UV Spectrum for Trametinib

Observation: While scanning the Trametinib solution we observed the maxima at 235nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Optimization of Chromatographic Conditions

The chromatographic conditions [5] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation [6] etc.

Table 3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Acetonitrile = 40 : 60	1.0ml/min	235nm	Very Low response	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Acetonitrile = 55 : 45	1.0ml/min	235nm	Low response	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Acetonitrile : Water = 50:50	1.0ml/min	235nm	Tailing peaks	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol : Water = 70:30	1.0ml/min	235nm	Resolution was not good	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 70:25:5	1.0ml/min	235nm	Tailing peak	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN : Methanol: 0.1% OPA = 60:30:10	1.0ml/min	235nm	Nice peak	Method accepted

Summary of optimized chromatographic conditions

The Optimum Chromatographic conditions [7] obtained from experiments can be summarized as below:

Table 4: Summary of Optimised Chromatographic Conditions

Mobile phase	ACN : Methanol: 0.1% OPA = 60:30:10
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	235 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	2.570 minutes

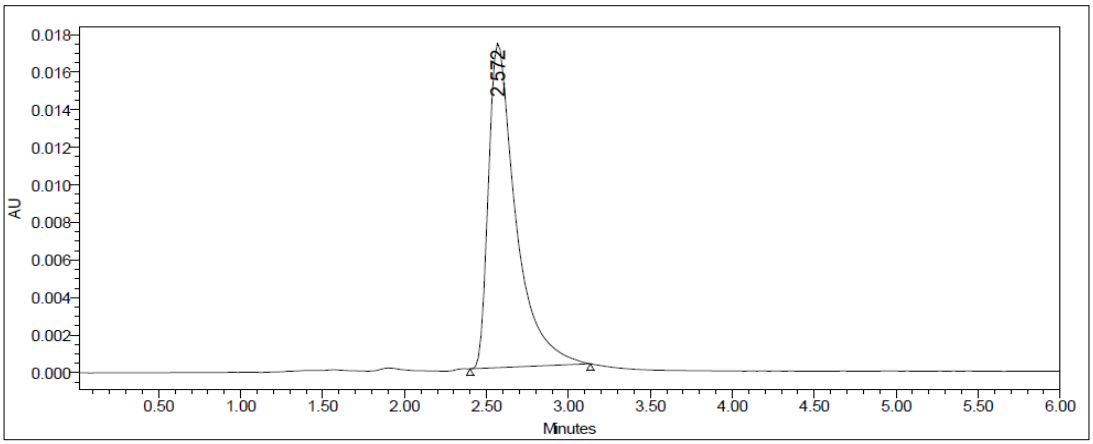

Fig 3: Chromatogram of Trametinib in Optimized Condition

Observation: The selected and optimized mobile phase [8] was ACN: Methanol: 0.1% OPA = 60:30:10 and conditions optimized were flow rate (1.0 ml/minute), wavelength (235nm), Run time was 06 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry [9]. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

Preparation of Mobile Phase

600ml of HPLC Grade Acetonitrile, 300ml of HPLC Grade Methanol and 100ml 0.1% OPA were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration [10].

Validation of Method

1. Accuracy:

Recovery study

To decide the exactness of the proposed strategy, recuperation thinks about were done by including diverse sums (80%, 100%, and 120%) of unadulterated medication of TRAMETINIB were taken and added to the pre-broke down plan of fixation 10µg/ml. From that rate recuperation [11] esteems were computed. The outcomes were appeared in table-5.

Table 5: Readings of Accuracy

Conc. In ppm	Conc. Found	Peak Area		% Recovery
8	8.035	161523		100.437
8	8.153	163815		101.912
8	8.061	162023		100.762
			Avg.	101.037
			S.D	0.775
			%RSD	0.767046
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
10	9.930	198315		99.30
10	10.033	200320		100.33
10	10.044	200540		100.44
			Avg.	100.0233
			S.D	0.628835
			%RSD	0.628688
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
12	11.981	238151		99.841
12	12.066	239819		100.55
12	12.215	242712		101.791
			Avg.	100.7273
			S.D	0.987021
			%RSD	0.979894

2. Precision

2.1. Repeatability

The precision [12] of each method was ascertained separately from the peak areas & retention times obtained by actual

determination of six replicates of a fixed amount of drug. Trametinib (API). The percent relative standard deviation [13] was calculated for Trametinib are presented in the table-6.

Table 6: Readings of Repeatability

HPLC Injection Replicates of Trametinib	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	2.572	197236
Replicate – 2	2.570	197762
Replicate – 3	2.573	195969
Replicate – 4	2.570	194724
Replicate – 5	2.574	198327
Replicate – 6	2.573	198711
Average		**197121.5**
Standard Deviation		**1515.213**
% RSD		**0.768667**

2.2. Intermediate Precision

2.2.1. Intra-assay & inter-assay

The intra & inter day variation [14] of the method was carried out & the high values of mean assay [15] & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Trametinib revealed that the proposed method is precise.

Table 7: Results of Intra-Assay & Inter-Assay

Conc. of Trametinib(API) (µg/ml)	Observed Conc. of Trametinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.46	0.62	8.05	0.96
10	10.87	0.85	9.43	0.71
12	11.81	0.92	12.04	0.65

3. Linearity & Range:

The calibration curve showed good linearity [16] in the range of 6 – 14 µg/ml, for Trametinib (API) with correlation coefficient [17] (r^2) of 0.999 (Fig-4). A typical calibration curve [18] has the regression equation of y = 19423x + 5444 for Trametinib.

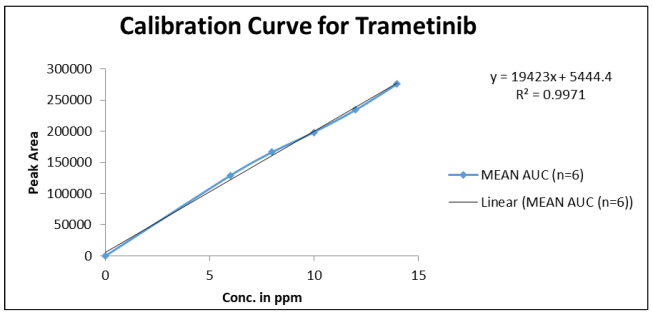

Fig 4: Calibration Curve of Trametinib (API).

Table 8: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
6ppm	129013
8ppm	166523
10ppm	198315
12ppm	234151
14ppm	275819

4. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase in mobile phase (±5%) studied to determine the robustness [19] of the method are also in favour of (Table-9, % RSD < 2%) the developed RP-HPLC method for the analysis of Trametinib (API).

Table 9: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.68
Flow (0.9 ml/min)	0.39
More Organic	0.54
Less Organic	0.63
Wavelength of Detection (237 nm)	0.91
Wavelength of detection (233 nm)	0.93

5. LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD [20]) & quantified (LOQ [21]) were found to be 0.08 & 0.24µg/ml respectively.

6. System Suitability Parameter: Framework appropriateness testing [22-26] is an essential piece of numerous scientific techniques. The tests depend on the idea that the gear, hardware, explanatory activities and tests to be broke down establish a vital framework that can be assessed all things considered. Following framework appropriateness test parameters were built up. The information is appeared in Table-10.

Table 10: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.47
2	Asymmetry	T ≤ 2	Trametinib=0.23
3	Theoretical plate	N > 2000	Trametinib=2987
4	Tailing Factor	T<2	Trametinib=1.17

7. Estimation of Trametinib in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms [27] were taken and the I.P. strategy was taken after to decide the normal weight. Above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused in five reproduces into the HPLC framework and the perceptions were recorded. A copy infusion of the standard arrangement was additionally infused into the HPLC framework [28] and the peak regions were recorded. The information is appeared in Table-11.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:
AT = Peak Area of medication acquired with test arrangement
AS = Peak Area of medication acquired with standard [29] arrangement
WS = Weight of working standard taken in mg
WT = Weight of test taken in mg
DS = Dilution of Standard arrangement
DT = Dilution of test arrangement
P = Percentage virtue of working standard

Table 11: Recovery Data for estimation Trametinib in Mekinist

Brand Name of Trametinib	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Mekinist Tablets (GlaxoSmithKline)	2mg	1.885 (±0.875)	99.89 (±0.452)

Result & Discussion: The amount of drug in Mekinist Tablets was found to be 1.885 (±0.875) mg/tab for Trametinib& % assay [30-31] was 99.89 %.

Summary and Conclusion

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Trametinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5µm, 15mmx4.6mm i.d. Column was preferred because using this column peak shape, resolution and absorbance were good.
Discovery wavelength was chosen in the wake of examining the standard arrangement of medication more than 200 to 400nm. From the U.V range of Trametinib it is apparent that a large portion of the HPLC works can be proficient in the wavelength scope of 210-300 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is amazingly, one more reasonable strategy for measure and dependability related debasement examines which can help in the investigation of Trametinib in various details.

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