Inter. J. PharmaO$_2$
ISSN: 2582-4708
Vol.7/Issue 2/2025; Patel P, et al., 78-88

International Journal of PharmaO$_2$
Journal Home Page: http://www.ijpo.in/
(IJPO: A Peer-reviewed Bi-monthly online journal)

Research Article

Develop And Validate Stability Indicating Analytical Method for Determination of Trametinib Using RP-HPLC

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ARTICLE INFO

Article history:
Received:; 01/09/2025
Revised:; 01/09/2025
Accepted:. 02/09/2025

Key Words:
Trametinib, RP-HPLC, Method Development, Validation.

Please cite this article as: *Patel P, et al.,* Develop And Validate Stability Indicating Analytical Method for Determination of Trametinib Using RP-HPLC, 7(2), 78-88.

ABSTRACT

To develop and validate a simple, accurate, economical, rapid, and precise RP-HPLC method for the determination of trametinib. Chromatographic separation was conducted on a YMC Pack ODS-A (150X4.6mm) 3.0 µ, 12 nm (Make: YMC) using a mobile phase of 0.01M Potassium Dihydrogen orthophosphate buffer: Acetonitrile (45:55 % v/v). The flow rate was set to 1.0 ml/min, and detection was performed at a wavelength of 243 nm. The retention time for trametinib was found to be 6.40 ± 0.05 minutes. The method was validated following ICH guidelines, assessing linearity, sensitivity, accuracy, precision, specificity, and robustness. The drug concentration range was 10-30 µg/mL, with a correlation coefficient of 1.0000. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.45 µg/mL and 1.38 µg/mL, respectively. The developed method exhibited high accuracy and precision, as indicated by satisfactory percentage recovery of trametinib. Statistical analysis confirmed precision and reproducibility of the results. The chromatographic conditions ensured effective resolution and precise quantification, making the method reliable for determining trametinib in pharmaceutical drug or formulations. The developed and validated RP-HPLC method is simple, rapid, and economical, providing accurate and precise determination of trametinib in bulk and dosage forms. It is suitable for routine analysis in pharmaceutical industries.

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Introduction:

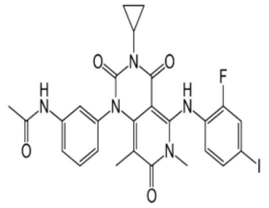
Malignant melanoma is the most aggressive of the cutaneous malignancies. For decades, the treatment options for patients with advanced disease were limited with an estimated median overall survival of less than a year (Balch et al., 2001). However, the treatment paradigm of metastatic melanoma has changed rapidly in recent years (Curti, 2014). Melanoma can be classified into distinct molecular cohorts based on the underlying genetic alterations. BRAFV600 mutations are seen in 40–50% of all melanomas. A substitution of valine for glutamine at this codon (V600E) occurs in nearly 90% of all BRAF mutant melanomas (Curtin et al., 2005; Lovly et al., 2012). Following BRAF, NRAS mutations are frequently noted in 15–25% of all melanomas (Fedorenko et al., 2013).

Melanoma can be classified into distinct molecular cohorts based on the underlying genetic alterations BRAFV600 mutations are seen in 40–50% of all melanomas. A substitution of valine for glutamine at this codon (V600E) occurs in nearly 90% of all BRAF mutant melanomas (Curtin et al., 2005; Lovly et al., 2012)

Following BRAF, NRAS mutations are frequently noted in 15–25% of all melanomas (Fedorenko et al., 2013). Other less common alterations include KIT mutations (1–2%), and mutations in GNAQ and GNA11 (80–90% of ocular melanomas) (Shtivelman et al., 2014). The remaining 30–35% of melanomas do not harbor recurrent alterations in these well-characterized driver genes. However, recently the NF1 mutated or deleted (a potential driver mutation) subset overall appears to represent up to 12% melanomas and is found within the BRAF-, NRAS-, CKIT- melanomas. Of note, certain mutations are notably associated with specific subtypes of melanomas such as KIT mutations seen in 15–20% of acral and mucosal melanoma (Curtin et al., 2006). Likewise, GNAQ and GNA11 mutations are seen in the large majority of uveal melanomas (Shoushtari & Carvajal, 2014; Van Raamsdonk et al., 2009).

Among several MEK inhibitors in clinical development, Trametinib (GSK1120212, Mekinist™, GlaxoSmithKline, London) is the only MEK 1/2 inhibitor approved by FDA either as monotherapy or in combination with dabrafenib for the treatment of unresectable or metastatic BRAFV600E or BRAFV600K mutant melanoma.

Properties of Drugs

Name of Drug	Trametinib
Group	Anti-Cancer Drug
Structure	
IUPAC Name	N-[3-[3-cyclopropyl-5-(2-fluoro-4-iodoanilino)-6,8-dimethyl-2,4,7-trioxopyrido[4,3-d]pyrimidin-1-yl]phenyl]acetamide
Molecular Weight	615.4 g/mol
Molecular Formula	$C_{26}H_{23}FIN_5O_4$
Solubility	Acetonitrile, Tetrahydrofuran, DMSO
Mechanism of Action	The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases (ERK) pathway, also known as the RAS-RAF-MEK-ERK pathway, activates a cascade of cell surface receptors and intracellular downstream signalling molecules. Activated RAS protein activates RAF, a serine/threonine kinase that activates other downstream proteins (Ho et al., 2014; Salama & Kim, 2013) such as mitogen-activated extracellular signal-regulated kinase 1 (MEK1)

	and MEK2. MEKs then activates ERK, which works on several target proteins and nuclear transcription factors that regulate cell proliferation, differentiation, survival, and growth.(Ho et al., 2014; Salama & Kim, 2013) ARAF, BRAF, and CRAF are three isoforms of RAF identified in humans. In particular, BRAF is known to be the most critical activator in melanoma. (Ho et al., 2014; Salama & Kim, 2013) Certain cancers, such as melanoma, are associated with BRAF mutations, with one study suggesting that BRAF is mutated in about 50% of melanoma tumours. (Ho et al., 2014) BRAF V600E and V600K mutations account for 95% of BRAF mutations. (Wright & McCormack, 2013) These BRAF mutations cause constitutive activation of the RAS-RAF-MEK-ERK pathway, leading to dysregulated proliferation and survival of tumour cells. (Wright & McCormack, 2013)

MATERIALS AND METHODS

Materials

Potassium dihydrogen phosphate was Purchased from Merck Ammonium acetate, Hydrochloric acid, Sodium Hydroxide, Dimethyl Sulfoxide were Purchased from Rankem Pvt Ltd. Ferric Chloride was Purchased Sigma-Aldrich Copper Chloride was purchased from Thermo-Scientific. Tetrahydrofuran and Hydrogen Peroxide were Purchased from SDFCL. All solvents were used from Analytical grade.

METHOD DEVELOPMENT

Selection of Solvents

The solubility of trametinib varies depending on the solvent: It has very low solubility in water, methanol and ethanol. In contrast, it exhibits solubility in tetrahydrofuran and dimethyl sulfoxide, slightly soluble in acetonitrile. Trametinib's solubility in water is much lower compared to other polar solvents such as alcohols.

Selection of Chromatographic Methods

The proper selection depends upon the nature of the sample, its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected.

HPLC Instrumentation

The HPLC system equipped with Waters Alliance HPLC System with Empower 3 Software built with Waters e2695 separation module connected with Waters 2489 UV-Visible Detector and Waters 2998 PDA Detector.

Mobile Phase Optimization

0.1M Ammonium acetate buffer

Weighed accurately and transferred about 7.7 grams of Ammonium acetate into a 1000 ml beaker and diluted to 1000 ml with water. Sonicate to dissolved and Filter through 0.45µm membrane filter paper [Millipore-

Durapore HVLP 0.45µm 047mm, CAT No. HVLP04700].

0.01M Potassium dihydrogen phosphate buffer

Weighed accurately and transferred about 1.36 grams of Potassium dihydrogen phosphate into a 1000 ml beaker and diluted to 1000 ml with water. Sonicate to dissolved and Filter through 0.45µm membrane filter paper [Millipore-Durapore HVLP 0.45µm 047mm, CAT No. HVLP04700].

Mobile phase

Buffer solutions were prepared and combined with acetonitrile in varying ratios to investigate the effects on chromatographic separation.

Diluent

Prepare a mixture of water and Acetonitrile in the ratio of 50:50 v/v and mix well. Degas it by sonication prior to use.

Column Selection

During method development such columns like YMC Pack ODS-A (150x4.6mm), 3.0µ (Make: YMC, Part No. AA12S03-1546WT, made in Japan) and Zorbax Eclipse XDB C-18 (150x4.6mm), 3.5µ (Make: Agilent, Part No. USWA018602, made in USA) are selected for better peak shape and chromatography.

Standard Preparation for Analysis

Standard preparation

Weigh accurately and transfer 25.00 mg of Trametinib standard into 100 ml volumetric flask, add 50 ml of acetonitrile. Sonicate for 10 to 15 minutes with intermittent shaking. Make up with water. Mix well and allow to attain solution at room temperature about 30 minutes. Further dilute by pipette out 5 ml standard stock preparation in to 50 ml volumetric flask and make up with diluent. (25 ppm of Trametinib)

Procedure

Inject the standard preparation in to the HPLC system by changing the chromatographic conditions, mobile phase as well as HPLC column and record the chromatograms, finalise the better chromatographic condition for performing validation parameters as per ICH guidelines.

Determination of λmax

Standard solution of Trametinib (20 µg/mL) was subjected to scanning between 200 and 400 nm on HPLC system equipped with Waters Alliance HPLC System with Empower 3 Software built with Waters e2695 separation module connected with Waters 2489 UV-Visible Detector and Waters 2998 PDA Detector.

Chromatographic condition selected for optimisation of method

Chromatographic analysis was performed on a column of YMC Pack ODS-A (150x4.6mm), 3.0µ (Make: YMC) and Zorbax Eclipse XDB C-18 (150x4.6mm), 3.5µ (Make: Agilent). The mobile phase consisted of methanol and water (10:90, v/v). The mobile phase was filtered and degassed through a 0.45 mm membrane filter before use and then pumped at a flow rate of 1 mL/min. The column oven temperature has been applied at 30 °C. Injection volume of sample selected 20 µl. The sample chiller storage temperature has been applied at 15 °C. The run time was 20 min under these conditions.

OPTIMISED CHROMATOGRAPHIC METHOD

Trametinib drug is basic and non-polar in nature. Based on this, columns and compositions of mobile phase was selected for retention of this drug. Proper peak shape with better system suitability parameters were obtained with mobile phase consisting of 0.01 M potassium dihydrogen phosphate buffer: acetonitrile in the ratio of (45:55) v/v. YMC Pack ODS-A (150x4.6mm), 3.0µ (Make: YMC, Part No. AA12S03-1546WT), column was selected. The flow rate was kept at 1.0 ml/minute and Wavelength maxima was carried out at 243 nm. Column oven temperature was maintained at 30˚C and sample temperature was maintained at 15˚C. Standard solution was prepared about 20 µg/ml of Trametinib. Standard solution was injected at 20 µl and the run time was 20 min under these conditions.

METHOD VALIDATION

Analytical Method validation was performed by ICH guideline recommendations for precision (system precision, method precision and intermediate precision), accuracy, specificity (force degradation), linearity, LOD-LOQ, robustness and Solution stability.

System suitability

The system suitability test was carried out by freshly prepared and inject standard preparation of Trametinib to check various parameters such as relative standard deviation, column efficiency (theoretical plates) and tailing factor. The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within ± 2.0 % relative standard deviation range during routine performance of the method.

Precision

Repeatability (Method Precision)

Five replicate injections of standard solution (20 ppm) and six sample preparations of same drug (20 ppm) were prepared and injected in duplicate into the chromatographic system. The mean % assay of trametinib solution was calculated and reported along with standard deviation and relative standard deviation of the six samples. The method precision was established by carrying out the analysis of Trametinib (n=6) using the proposed method.

Ruggedness (Intermediate Precision)

Intermediate Precision was performed by making five replicates of standard solution (about 20 ppm of trametinib) and six replicates of sample preparations of trametinib (about 20 ppm) and injected on two different days and on different instruments.

Specificity / Force Degradation

Specificity of the method was carried out by injecting blank and sample solution (20 ppm), there were no interference observed at the retention time of Trametinib peak. The force degradation study of the method can be demonstrated by applying stress conditions using 1M Hydrochloric acid, 1M Sodium hydroxide, 3% Hydrogen peroxide, Wet and Dry thermal degradation, Photolytic Degradation, Humidity degradations. The Trametinib drug solution was subjected to these conditions, and the principal peak of the drug was analysed for peak purity, suggesting that the approach efficiently separated the degradation products from the pure active ingredient. Peak purity criteria is peak angle should be less than peak threshold.

Linearity

The linearity was carried out by injecting the series of standard preparation of trametinib between the concentration range of 50 % to 150 % of working concentration. A linear plot with concentration of drug on X-axis and the corresponding mean peak area on Y-axis was plotted.

Limit of Detection and Limit of Quantitation

Calculated based on relation of Standard deviation of Response and Slope obtained from linearity.

The limit of detection and Limit of quantification calculated based on the following equation.

LOD= 3.3X {Standard deviation of Response / Slope} and LOQ= 10 X {Standard deviation of Response / Slope}.

Calculated based on % RSD and S/N ratio.

A series of standard preparation of different concentration of Trametinib were prepared by diluting linearity preparation-50% and injected in to HPLC in triplicate injection. The % RSD and USP S/N ratio were calculated.

Robustness

Standard preparation was analysed by changing in chromatographic condition of method. The changes include different flow rates of the mobile phase (±0.2 mL/min), acetonitrile ratio in the mobile phase (±10%), wavelength (±2.0 nm) and column temperatures (±5ºC). After each change, system suitability parameters were checked by injecting the standard solution into the chromatographic system and the results were compared with the method precision.

Solution stability studies

This study was evaluated by injecting freshly prepared standard and sample preparation at different time interval at 15°C sample chiller temperature.

RESULTS AND DISCUSSION

Objective of this work is an estimation of Trametinib by RP-HPLC method is use to determine Assay of trametinib in quality control of the raw material, formulation as well as dissolution studies. The analytical method was developed by applying different parameters. First of all, maximum absorbance was found to be at 243 nm, Fig. 1. In the proposed HPLC method, different combination ratios of the buffer preparation and acetonitrile were attempted at flow rate of 1 mL/min. The ratio of 0.01 M Phosphate buffer: Acetonitrile (45:55 v/v) was fixed due to better symmetrical peak. Injection volume was selected 20 µl which gave a better peak shape and chromatography. The column was selected YMC Pack ODS A (150X4.6mm) 3.0 µ, 12 nm because it gave proper peak. Column oven temperature was selected 30°C. The flow rate was fixed at 1.0 ml/min for well peak area and satisfactory retention time. Water: Acetonitrile was selected for completely soluble and showed good recovery. Retention time of Trametinib obtained around 6.45 min based on that run time was selected 20 min.

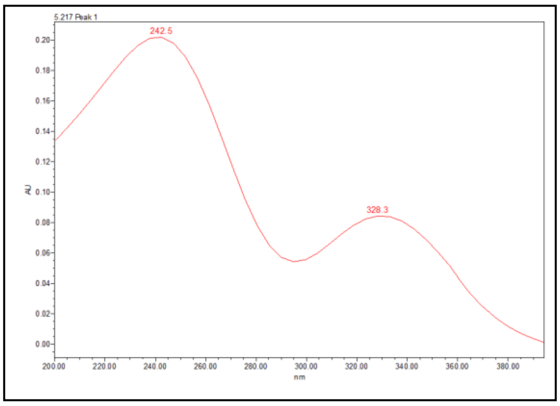


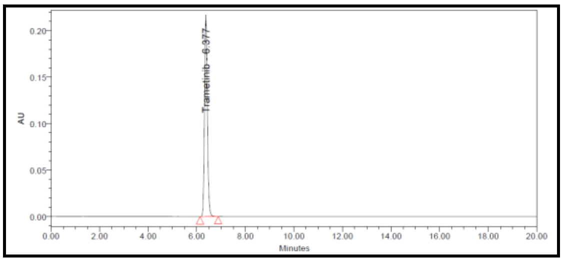
Fig. 1: Scan in PDA Spectrum of Trametinib

System suitability

The system suitability of the method was established by five replicate injections of the standard solution containing of 20 ppm of Trametinib solution. Relative standard deviation of five replicate injections, USP plate counts and USP tailing values are mentioned in Table-1. Based on the peak results, the developed analytical method shows satisfactory value for system suitability, and the standard chromatogram was shown in Fig-2.

Table-1: System suitability parameters of Trametinib

No. of Injection	**Area counts of Trametinib**	**Retention Time**	**Height (µV)**	**Theoretical Plates**	**Tailing Factor**
1	1743022	6.38	212332	13677	1.14
2	1743618	6.38	212333	13707	1.14
3	1744913	6.38	212472	13650	1.14
4	1747480	6.37	213880	13722	1.15
5	1744496	6.38	212448	13679	1.14
Mean	**1744706**	**6.38**		-	-
SD	**1717.67**	**0.0045**		-	-
RSD	**0.10**	**0.10**		-	-



	Name	Retention Time	Area	% Area	Height	USP Resolution	USP s/n	USP Tailing	USP Plate Count
1	Trametinib	6.377	1743022	100.00	212332		20507	1.14	13677

Fig. 2: System suitability of standard preparation of Trametinib

Precision

Repeatability (Method Precision)

Method precision of this analytical method obtained from five replicate injections of standard solution (20 ppm) and six sample preparations of same drug (20 ppm) were prepared and injected in duplicate into the chromatographic system. The results shown in Table 2.

Table 2: % Assay of Trametinib in repeatability study

No. of Sample preparation	Sample Weight (mg)	Concentration (ppm)	Mean Area of two injections	% Assay of Trametinib	% Deviation from mean Assay
Sample -1	25.06	20.0480	1765222	101.66	0.51
Sample -2	25.09	20.0720	1763567	101.44	0.30
Sample -3	25.13	20.1040	1746798	100.32	-0.81
Sample -4	25.09	20.0720	1747437	100.52	-0.62
Sample -5	25.24	20.1920	1751726	100.16	-0.97
Sample -6	25.30	20.2400	1755197	100.12	-1.01
Between 98 % to 102 %			**Mean**	**101.14**	**(Limit±2.0%)**
			SD	**0.720**	
NMT 2.0%			**RSD**	**0.70**	

Ruggedness (Intermediate Precision)

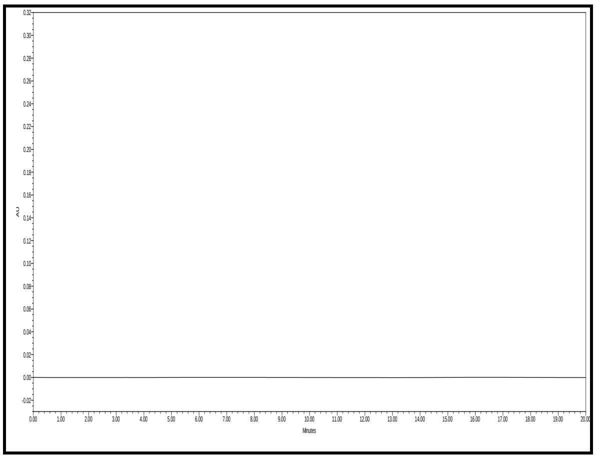
Intermediate Precision was performed on two different days and on different instruments. By injecting five replicates of standard solution (about 20 ppm of trametinib) and six replicates of sample preparations of trametinib (about 20 ppm). The results shown in Table 3.

Table 3: % Assay of Trametinib in ruggedness study

No. of Sample preparation	Sample Weight (mg)	Concentration (ppm)	Mean Area of two injections	% Assay of Trametinib	% Deviation from mean Assay
Sample -1	25.16	**20.1280**	**1754531**	**100.82**	**0.31**
Sample -2	25.02	**20.0160**	**1726796**	**99.78**	**-0.72**
Sample -3	25.10	**20.0800**	**1752229**	**100.92**	**0.42**
Sample -4	25.15	**20.1200**	**1743623**	**100.23**	**-0.28**
Sample -5	25.00	**20.0000**	**1762915**	**101.95**	**1.43**
Sample -6	25.05	**20.0400**	**1745318**	**100.73**	**0.22**
Between 98 % to 102 %			**Mean**	**100.51**	**(Limit±2.0 %)**
			SD	**0.633**	
NMT 2.0%			**RSD**	**0.60**	

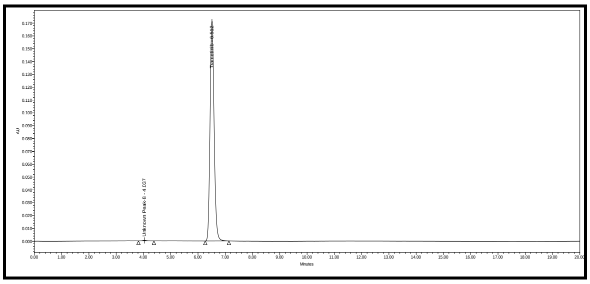
Specificity / Force Degradation

Specificity of the method was carried out by injecting blank and sample solution (20 ppm). No interference observed at the retention time of Trametinib peak due to blank and mobile phase. The Trametinib drug solution was subjected to stress conditions by adding degradant reagent as well as applied heat, light and moisture condition, and the principal peak of the drug was found pure, indicating that method is specific. The results shown in Table 4 and chromatograms were shown in Fig-3 and 4 respectively.



	Name	Retention Time	Area	% Area	Height	USP Resolution	USP s/n	USP Tailing	USP Plate Count
1	Trametinib	6.377							

Fig. 3: Chromatogram of Blank preparation



	Name	Retention Time	Area	% Area	Purity1 Angle	Purity1 Threshold	Height	USP Resolution	USP s/n	USP Tailing	USP Plate Count
1	Unknown Peak-8	4.037	3623	0.21	8.582	4.231	390		22	1.17	4572
2	Trametinib	6.512	1760141	99.79	0.023	0.206	171318	9.70	10042	1.15	9194

Fig. 4: Chromatogram of Sample preparation

Table 4: % Assay of Trametinib in force degradation study

Sr. No.	Stress Condition	Peak Area	% Assay	% Degradation	Peak Angle	Peak Threshold
1.	Sample as such	1760141	99.89	0.00	0.023	0.206
2.	Acid degradation	1666660	94.58	-5.31	0.031	0.209
3.	Alkali-Optimised	1720374	97.63	-2.26	0.027	0.211
4.	Alkali degradation	793813	45.05	-54.90	0.055	0.227
5.	Peroxide degradation	1731656	98.27	-1.62	0.026	0.209
6.	Wet thermal degradation	1749011	99.26	-0.63	0.027	0.217
7.	Dry thermal degradation	1729435	97.95	-1.94	0.031	0.208
8.	Metal-$CuCl_2$ degradation	1353946	76.84	-23.08	0.184	0.219
9.	Metal-$FeCl_3$ degradation	1715190	97.34	-2.55	0.023	0.211
10.	Photolytic degradation	1739471	97.94	-1.95	0.027	0.205
11.	Humidity degradation	1775573	99.74	-0.15	0.026	0.234

Linearity

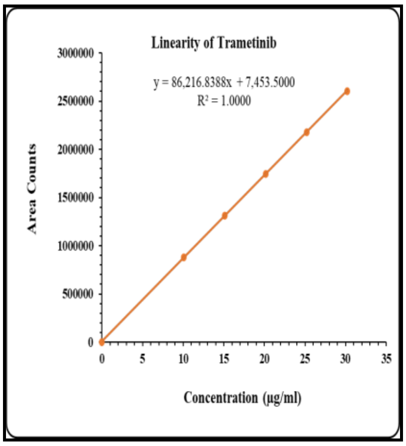
The linearity was performed by injecting the series of standard preparation of trametinib between the concentration range of 50 % to 150 % of working concentration range between 10 and 30 µg/ml. Calibration curve shows in Fig. 5. The correlation coefficient for trametinib was found to be 1.0000. Based on the data, trametinib drug response is confirmed to be linear from 10-30 µg/ml. The linearity dilution shown in Table 5 and results shown in Table 6.

Table 5: Linearity dilution

Linearity Preparation	Volume of standard stock solution (ml)	Final Dilution (ml)	Concentration (µg/ml)
Linearity-50%	2	50	10.07
Linearity-75%	3	50	15.11
Linearity-100%	4	50	20.14
Linearity-125%	5	50	25.18
Linearity-150%	6	50	30.22

Table 6: Linearity of trametinib

Linearity Preparation	Concentration (µg/ml)	Average peak area counts
Linearity-50%	10.07	883036
Linearity-75%	15.11	1313322
Linearity-100%	20.14	1746255
Linearity-125%	25.18	2180278
Linearity-150%	30.22	2605591
Slope		**86216.8388**
Intercept		**7453.5000**
Correlation Coefficient (r)		**1.0000**



Limit of Detection and Limit of Quantitation

A. Calculated based on relation of Standard deviation of Response and Slope obtained from linearity.

The LOD for Trametinib was confirmed to be 0.45 µg/ml and LOQ for Trametinib was confirmed to be 1.38 µg/ml.

B. Calculated based on % RSD and USP S/N ratio.

LOD and LOQ were determined based on the peak response of trametinib by injecting diluted standard solution of trametinib. The % RSD and USP S/N ratio were calculated. Based on the data, LOQ for trametinib is 0.100 µg/ml and LOQ for trametinib is 0.050 µg/ml. The results shown in Table 7.

Table 7: LOD-LOQ determination

LOD-LOQ Level	Concentration (µg/ml)	Average Area counts of 3 injections	% RSD of 3 injections	USP S/N
LOD-LOQ-1	1.0000	89453	0.30%	537
LOD-LOQ-2	0.5000	44779	0.20%	1741
LOD-LOQ-3	0.2000	17840	0.40%	404
LOD-LOQ-4	0.1000	9106	1.70%	449
LOD-LOQ-5	0.0500	5572	10.0%	191

Robustness

In Robustness, system suitability parameters were within acceptance and results were compared with the method precision. From the data of robustness, it was observed that there was no major impact in the chromatograms, which indicate that the RP-HPLC method developed is robust. The results mentioned in Table 8.

Robustness parameters	Retention time	Area	Theoretical Plates	Tailing Factor
Normal condition	6.38	1743022	13677	1.14
Increase Flow rate i.e. 1.2 ml/min	5.37	1460530	12466	1.13
Decrease Flow rate i.e. 0.8 ml/min	7.92	2196959	14854	1.16
Increase Column Temperature i.e. 35°C	6.26	1758094	13730	1.15
Decrease Column Temperature i.e. 25°C	6.47	1758236	13519	1.15
Increase Wavelength i.e. 245 nm	6.46	1733158	9605	1.13
Decrease Wavelength i.e. 241 nm	6.46	1625831	9604	1.13
Increase Organic Ratio i.e. Buffer: Acetonitrile (40:60)	4.90	1775070	11568	1.18
Decrease Organic Ratio i.e. Buffer: Acetonitrile (50:50)	9.29	1784364	15130	1.13

Solution stability studies

Solution stability data indicate that standard preparation and sample preparation were stable up to 62 hours and 57 hours respectively at 15°C in analytical solution. % Deviation and % RSD between initial and specified time interval area count found within acceptance criteria. The results mentioned in Table 9 and 10.

Table 9: Solution stability of standard preparation

Solution stability of Standard solution			
Time (Hrs)	**Area Counts of Trametinib**	**% Deviation from mean Assay (Limit±3.0%)**	**% RSD Between Initial and Specified Interval Area Count (Limit±2.0%)**
0	1743022	0.00	0.00
6	1747929	0.28	0.20
11	1750322	0.42	0.30
18	1759145	0.93	0.65
26	1762888	1.14	0.80
37	1768340	1.45	1.02
44	1769254	1.50	1.06
52	1783872	2.34	1.64
62	1787160	2.53	1.77

Table 10: Solution stability of sample preparation

Solution stability of Standard solution			
Time (Hrs)	**Area Counts of Trametinib**	**% Deviation from mean Assay (Limit±3.0%)**	**% RSD Between Initial and Specified Interval Area Count (Limit±2.0%)**
0	1763424	0.00	0.00
5	1763872	0.03	0.02
12	1768250	0.27	0.19
21	1767293	0.22	0.15
31	1777902	0.82	0.58
39	1778476	0.85	0.60
46	1786261	1.30	0.91
57	1793383	1.70	1.19

CONCLUSION

System precision and method precision found to be accurate and well within acceptance criteria. The % recovery found between 98.0 % - 102.0 % over the same range of working concentration. The analytical method was found linearity over the range of 10-30 ppm of the Trametinib target concentration. The analytical method shows robustness and ruggedness also well satisfactory. Standard solution of trametinib also stable more than 2 days in selected diluent. The proposed RP-HPLC method for the estimation of Trametinib is accurate, precise, specific, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the use to determine Assay of trametinib in quality control of the raw material, formulation as well as dissolution studies.

CONFLICT OF INTERESTS

The authors of this manuscript do not have any financial or no-financial conflicts of interest.

ACKNOWLEDGEMENT

The authors are thankful to Sun pharmaceutical research centre and Saurashtra university for providing facilities in this research work.

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