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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF TRAMETINIB IN API AND MARKETED PHARMACEUTICAL TABLET DOSAGE FORM

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Abstract:

It has been developed a precise, right, rough, and precise switched fragment over the top execution fluid chromatography (RP-HPLC) technique to quantify the quantitative commitment of Trametinib in active drug parts and in its Pharmaceutical dosage structure by using the use of the C18 (four.6mm x 150m, 5m) column with the cell segment containing an Acetonitrile and Potassium dihydrogen phosphate cushion acclimated to pH-2.8 with ortho phosphoric acid in the cell section. The float charge was increased to 1.0 ml/min, gushingwas seen at 246 nm, a pinnacle eluted at 4.865 minutes, and the section broiler temperature was maintained at the surrounding level. A range of 10 to 30 g/ml was indicated on the adjustment bend. Trametinib's LOD and LOQ were reported to be 1.three and 3.9 micrograms per millilitre, respectively. There is no guarantee that the offer recovery will fall inside the cutoff thresholds. Extensiveness, LOD, LOQ, linearity, exactness, precision, middle of the road accuracy, and power were all found to be consistent with the currentglobal gathering on Harmonization (ICH) concepts for these parameters. Trametinib typical resolution in mass medicine and its drug dosage form may be helped by the suggested RP- HPLC technique, which was shown to be easy to use, rapid, accurate, and precise. The suggested method was used to examine tablet designs for quality control and assurance of remedial feasibility, as outlined in the paper.

Keywords: *Trametinib, RP-HPLC, Accuracy, Precision, Validation, and ICH.*

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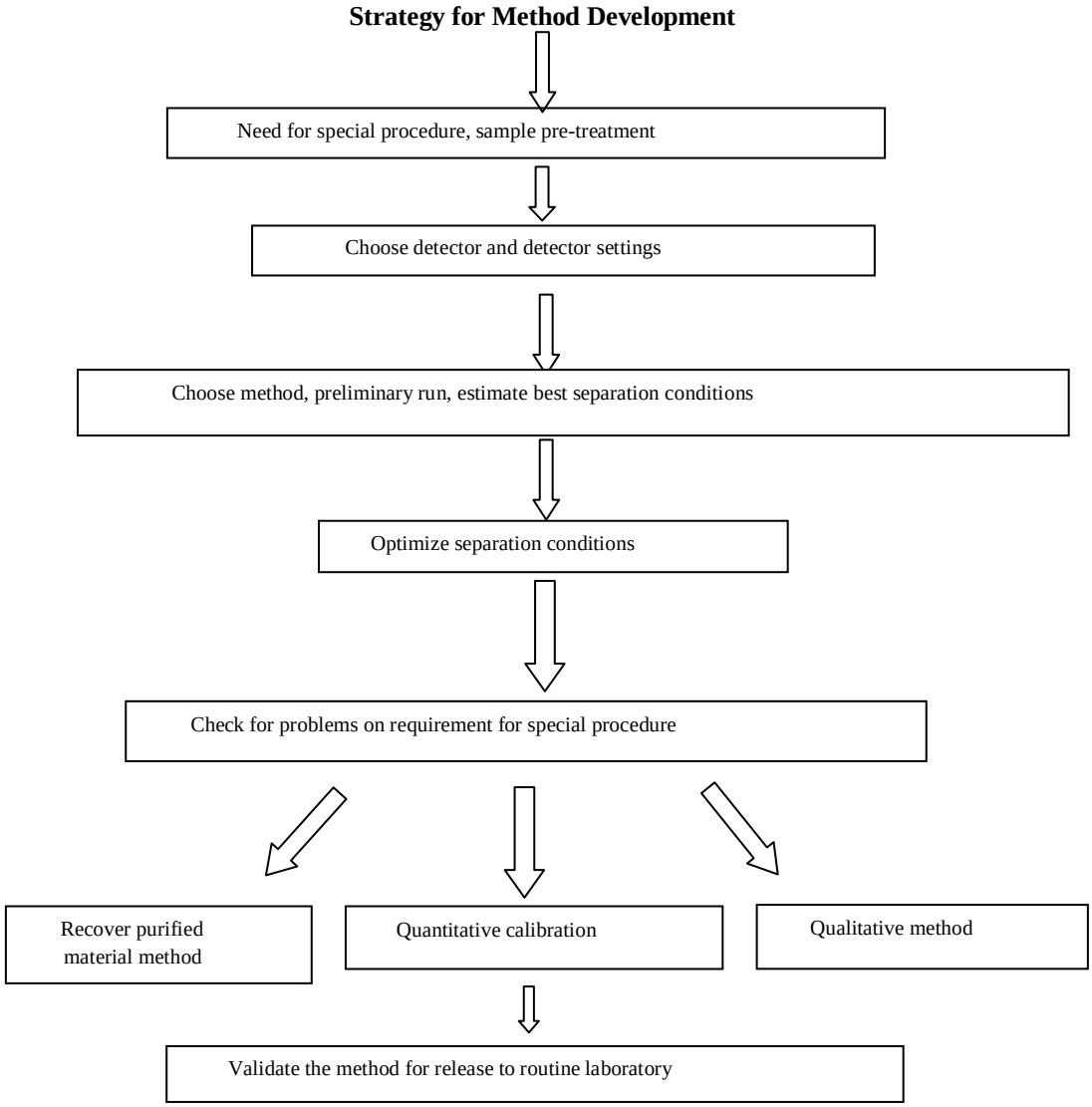
INTRODUCTION:

Pharmaceutical analysis, a specialized branch of analytical chemistry plays an important role in quality assurance & quality control of bulk drugs and their formulation. Where method of analysis is routinely developed, validated & collaboratively studied and applied. Methods of drug estimation done by physical,chemical & physicochemical methods[1]. Chromatographic technique is a differential migration of components takes place between two phases, one is stable & another is mobile known as stationary phase & mobile phase respectively. This technique is predominantly used in pharmaceutical industry for analyzing variety of samples. HPLC is one of them used widely for checking the purity & evaluation of new drug candidates & quality control assurance of final drug products [2].

Table-1: Commonly used columns for method development and validation

S.NO.	MFG COMPANEY	COLUMN BRAND NAME	PARTICAL SIZE (µm)	COLUMN DIMENSIONS (mm)	TYPE OF COLUMN
1.	Waters	X-Terra	5 µm	4.6(i.d)×250mm	C18
2.	ACE	Ace HPLC Column	5 µm	4.0(i.d)×250mm	C18
3.	Lichrochart	Lichrospher	5 µm	4.0(i.d)×250mm	C18
4.	GL Sciences	Inertsil ODS	5 µm	4.6(i.d)×250mm	C18
5.	Lmtakt	Unison US	5 µm	4.6(i.d)×250mm	C18
6.	Waters	Symmetry Shield	5 µm	4.6(i.d)×250mm	C18
7.	Waters	Spherisorb	5 µm	4.6(i.d)×250mm	C18
8.	Zodiac Life Sciences	Zodiac	5 µm	4.6(i.d)×250mm	C18
9.	Nomura Chemical	Develosil ODS	5 µm	4.6(i.d)×250mm	C18
10.	Grace	Kromasil	5 µm	4.6(i.d)×150mm	C18
11.	Thermo	Hypersil BDS	5 µm	4.6(i.d)×150mm	C18
12.	Waters	X-Terra	5 µm	4.6(i.d)×150mm	C8

Figure-1: Flow Chart of HPLC Method Development



Getting Started On Method Development

One approach is to use an isocratic mobile phase of some average organic solvent strength (50%). A better alternative is to use a very strong mobile phase first (80-100%) then reduce %B as necessary. The initial separation with 100% B results in rapid elution of the entire sample but few groups will separate. Goals that are to be achieved in method developmentare briefly summarized.

Table-2: Goals of Method Development

Goal	**Comment**
Separation time	<5-10 min is desirable for routine procedures.
Quantitation	□2% for assays; □5% for less-demanding analyses □15% for trace analyses.
Pressure	<150 bars is desirable, <200 bars is usually essential (new column assumed).
Peak height	Narrow peaks are desirable for large signal/noise ratios.
Solvent consumption	Minimum mobile-phase use per run is desirable.

Optimization of HPLC method

During the optimization stage, the initial sets of conditions that have evolved from thefirst stages of development are improved or maximized in terms of resolution and peak shape,plate counts, asymmetry, capacity factor, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analyte of interest.

Table-3: Different Buffers used in HPLC Method Development

Buffer	pKa(25°C)	Maximum Buffer Range	UV Cutoff (nm)
TFA	0.3	--	210 (0.1%)
Phosphate, H_2PO_4 pK_1	2.1	1.1-3.1	< 200
Phosphate, pK_2 HPO_4^{-2}	7.2	6.2-8.2	< 200
Phosphate, pK_3 PO_4^{-3}	12.3	11.3-13.3	< 200
Citrate, pK_1 $C_3H_5O(CO_2H)_2(CO_2\text{-})_1$	3.1	2.1-4.1	230
Citrate, pK_2 $C_3H_5O(CO_2H)_1(CO_2\text{-})_2$	4.7	3.7-5.7	230
Citrate, pK_3 $C_3H_5O(CO_2\text{-})_3$	6.4	4.4-6.4	230
Carbonate, pK_1 HCO_3^{-2}	6.1	5.1-7.1	< 200
Carbonate, pK_2 CO_3^{-2}	10.3	9.3-11.3	> 200
Formate	3.8	2.8-4.8	210 (10nM)
Acetate	4.8	3.8-5.8	210 (10nM.)
Ammonia	9.3	8.3-10.3	200 (10nM)
Borate	9.2	8.2-10.2	N / A
TEA	10.8	9.8-11.8	< 200

Table-4: Optimization Parameters in HPLC Method Development

Analytes	HPLC method	Optimize
Neutral	Reverse phase	Solvent strength, solventtype
Weak acids and/orweak bases	Ion suppression	pH, solvent strength,solvent type
Strong acid and/orstrong bases	Ion pairing	Ion pairing reagentconcentration, pH solvent strength , solvent type
Inorganic anions/cations	Ion exchange	Eluting ion concentration

METHOD VALIDATION:
Method validation study include system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection, limit of quantification and stability of samples, reagents, instruments.

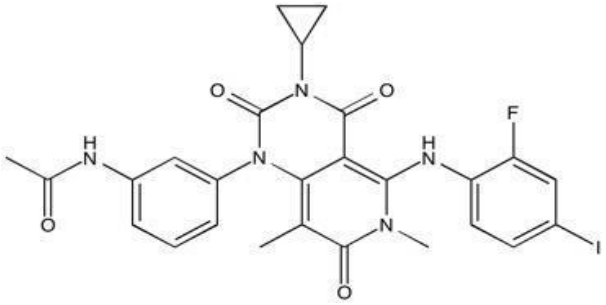
DRUG PROFILE DRUG PROFILE FOR TRAMETINIB

Name of the drug: Trametinib

Description: Trametinib is an orally bioavailable inhibitor of mitogen-activated protein kinase (MEK MAPK/ERK kinase) with potential antineoplastic activity. Trametinib specifically bindsto 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 threonine/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.

Synonyms: Trametinib, 871700-17-3, GSK1120212, Mekinist, GSK 1120212 and Trametinibum.

Chemical Structure:



IUPAC Name: N-(3-{3-cyclopropyl-5-[(2-fluoro-4-iodophenyl) amino]-6,8-dimethyl-2,4,7- trioxo-1H,2H,3H,4H,6H,7H-pyrido[4,3-d] pyrimidin-1-yl} phenyl) acetamide.

Molecular Formula: $C_{26}H_{23}FIN_5O_4$ Molecular weight: 615.3948g/mole PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): Trametinib is a crystalline solid powder.

Solubility: Trametinib was found to be soluble in DMSO at 20 mg/mL with warming; very poorly soluble in ethanol; very poorly soluble in water; maximum solubility in plain water is estimated to be about 5-10 µM; buffers, serum, or other additives may increase or decrease the aqueous solubility, soluble in organic solvents such as DMSO and dimethyl formamide, Acetonitrile.
Storage Conditions: Store at controlled room temperature of 20°C to 25°C (68°F to 77°F). Brief (less than 24 hours) exposure to temperatures up to 30°C (86°F) is acceptable.
Dosage: Tablet, film coated. Melting point: 293°C - 303°CpKa (Strongest Acidic): 12.6Log P: 3.18
Pharmacokinetics:

Bioavailability: A single 2 mg oral dose has a bioavailability of 72%.

Absorption: Trametinib is readily absorbed. When an oral administration of Trametinib was given to patients with BRAF V600 mutation-positive melanoma, peak plasma concentration occurred 1.5 hours post-dose (Tmax). A single 2 mg oral dose has a bioavailability of 72%. When a dose of 2mg/day is given, the peak plasma concentration (Cmax) is 22.2ng/mL

Volume of distribution: Apparent volume of distribution (Vd/F) = 214 L.

Protein binding: 97.4% bound to human plasma proteins.

Metabolism: Trametinib is metabolized predominantly via deacetylation alone or with mono-oxygenation or in combination with glucuronidation biotransformation pathways in vitro. Deacetylation is likely mediated by hydrolytic enzymes, such as carboxyl-esterases or amidases. The cytochrome P450 enzyme system is not involved with the metabolism of Trametinib. The predominant circulating component in the plasma is the parent drug.

Route of Elimination: 80% of the dose is excreted in the feces. <20% of the dose is excreted in the urine with <0.1% of the excreted dose in the form of the parent compound.

Half Life: Elimination half-life = 3.9-4.8 days.

Pharmacodynamics: 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.

Mechanism of Action: Trametinib 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.

Drug Interactions: drugs like acarbose, aceclofenac, abacavir & acemetacin found to decrease trametinib excretion rate resulting in higher serum level.

Drug-Food Interactions: Take separate from meals. Take at least one hour before or two hours after a meal.

Contraindications: Dehydration, fever, hypotension, renal failure, serious fever and febrile reactions including symptoms of hypotension, rigors/chills, dehydration, and/or renal failure have been reported in patients who received Trametinib in combination with Dabrafenib.

Adverse effects/Side Effects: Commonly reported side effects of Trametinib include: acneiform eruption, cardiomyopathy, decreased left ventricular ejection fraction, dermatitis, palmar-plantar erythrodysesthesia, skin rash, and erythema of skin. Other side effects include: cardiac failure.

Medical Uses: Trametinib is a cancer medicine that interferes with the growth and spread of cancer cells in the body. Trametinib is used alone or in combination with another medicine called Dabrafenib (Tafinlar) to treat certain types of cancer in people who have a "BRAF" genemutation.

Table no: 5 MARKETED FORMULATION

S.NO.	Drug Name	Brand Name	Label Claim	Company Name
1	Trametinib	Mekinist Tab	0.5,1 and 2mg	GlaxoSmithKline

Table no: 6 LITERATURE REVIEW

s.no	year	author	Method development
1.	**2018**	**NVS Naidu, et al**	**Method achieved by using isocratic solution** with ratio ammonium acetate buffer: methanol: Acetonitrile: tetrahydrofuran mobile phase at 40:30:20:10V/V.
2.	**2018**	**Kafiya Suroor, et al.**	In this method, separation and assay of Encorafenib and Binimetinib was done in stationary phase using Agilent C18 column with mobile phase of 0.1M dipotassium hydrogen phosphate (pH 4.0) and methanol in 50:50 vol/vol ratio.
3.	**2016**	**Cynthia Marcella Nijenhuis, et al.**	To support therapeutic drug monitoring (TDM) and clinical pharmacological trials, an assay to simultaneously quantify Dabrafenib and Trametinibin human plasma using liquid chromatography tandem mass spectrometry was developed and validated.
4.	**2014**	**Kevin Bihan, et al.**	A stability study of vemurafenib in human plasma was performed by the methods using (13) C (6)-vemurafenib as the internal standard.

AIM & OBJECTIVE: The present work is undertaken with an objective to develop economical, simple, precise, accurate and reproducible assay method for the estimation of Trametinib in bulk and their single and combined marketed tablet dosage form by RP-HPLC.

PLAN OF WORK

1. **Literature Survey**
2. **Procurement of selected drug for Study:** Reference standards were procured from pharmaceutical company.
3. **Selection of tablet formulation:** By market survey and literature.
4. **Selection of analytical technique:** HPLC

A) Estimation of drugs in combination by RP-HPLC methods:

1) Selection of column.
2) Selection and optimization of Mobile phase.
3) Selection of different chromatographic conditions.
4) System suitability parameters study.
5) Analysis of standard laboratory mixture to see feasibility of proposed method.
6) To adopt selected method on marketed formulation.
7) Validation of Proposed Methods.
   - Accuracy
   - Precision
   - Specificity
   - Linearity
   - LOD/LOQ
   - Ruggedness
   - Robustness

METHOD DEVELOPMENT:

Instruments used

Table no: 7: Instruments used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module,Software: Empower 2, 996 PDA Detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED:

Table no: 8: Chemicals used

S.No	Chemical	Brand names
1	Trametinib (Pure)	Sura labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck
4	Potassium Dihydrogen Phosphate	Finar Chemicals

HPLC METHOD DEVELOPMENT:

Trials

Trial 1:

Column	:	Inertsil C18 (4.6 x 250mm, 5□m)
Column temperature	:	45ºC
Wavelength	:	246 nm
Mobile phase ratio	:	Acetonitrile: Water (55:45 % v/v)
Flow rate	:	1.0mL/min
Injection volume	:	10 µl
Run time	:	10minutes

Figure no: 2 Chromatogram for Trial 1

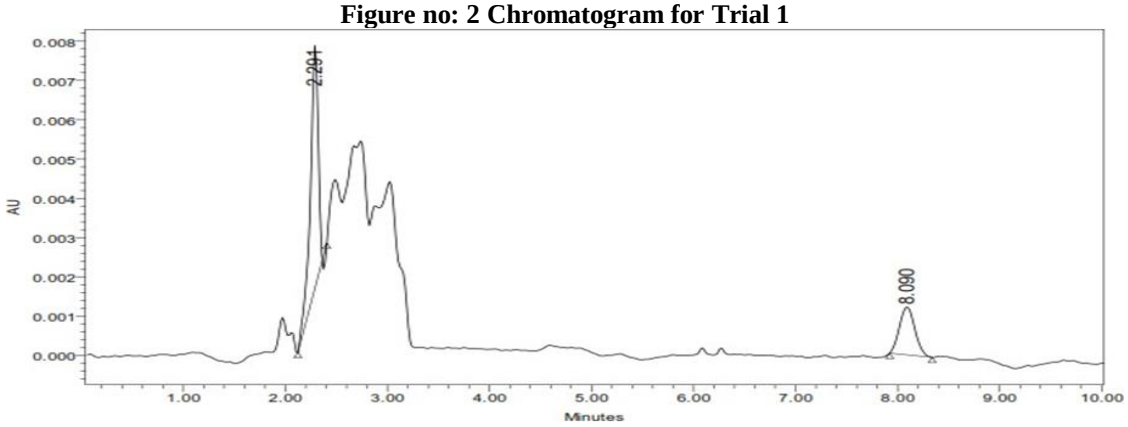


Table no: 9: Peak Results for Trail 1

S.No.	Peak Name	Rt	Area	Height	USP Tailing	USP Plate count
1	Trametinib	2.291	245523	25416	0.95	1154

Observation: The trial shows more void peaks and improper baseline in the chromatogram. So more trials were required for obtaining peaks.

Trial 2:

Column	:	Inertsil C18 (4.6 x 150mm, 5□m)
Column temperature	:	30ºC
Wavelength	:	246 nm
Mobile phase ratio	:	Methanol: Water (70:30)
Flow rate	:	1.0mL/min
Injection volume	:	20 µl
Run time	:	9 minutes

Figure no: 3 Chromatogram for Trial 2

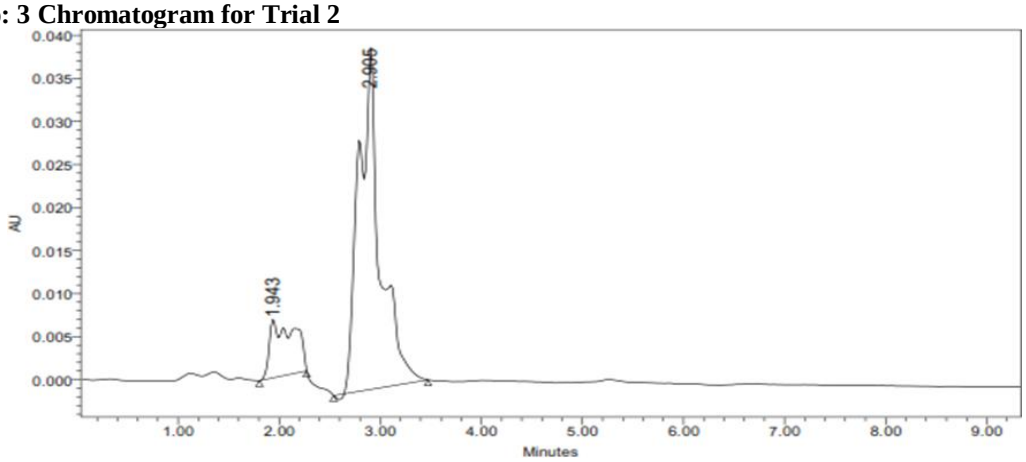


Table no: 10: Peak Results for Trial 2

S. No.	Peak name	Rt	Area	Height	USP Tailing	USP plate count
1	Trametinib	2.995	365881	54871	2.55	2548

Observation: This trial show more void peaks and improper baseline in the chromatogram, so more trials were required for obtaining peaks.

Trial 3:

Column	:	Zorbax C18 (4.6 x 150mm, 5□m)
Column temperature	:	40^0C
Wavelength	:	246 nm
Mobile phase ratio	:	Methanol: Acetonitrile (70:30)
Flow rate	:	1.0 mL/min
Injection volume	:	20 µl
Run time	:	8 minutes

Figure no: 4 Chromatogram for Trial 3

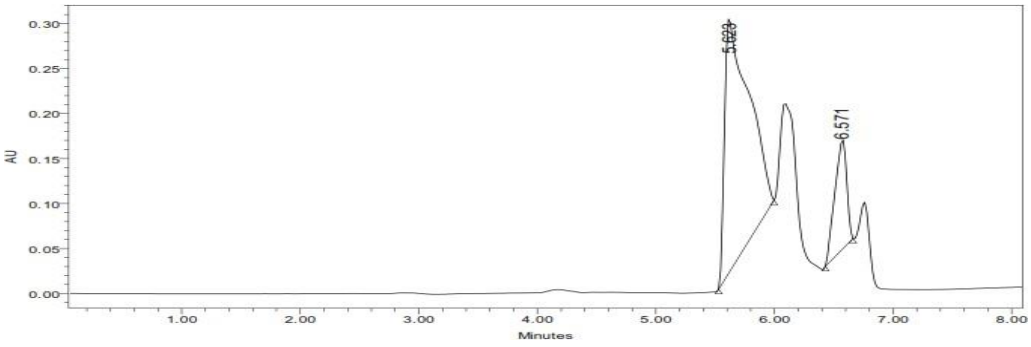


Table no: 11: Peak Results for Trial 3

S. No.	Peak name	Rt	Area	Height	USP Tailing	USP plate count
1	Trametinib	5.623	458745	52641	3.45	1215

Observation: This trial show more tailing and improper baseline and peak in the chromatogram, so more trials were required for obtaining good peaks.

Trial 4:

Column	:	Develosil C18 (4.6 x 150mm, 5□m)
Column temperature	:	35^0C
Wavelength	:	246 nm
Mobile phase ratio	:	Methanol: Acetonitrile (40:60)
Flow rate	:	1 mL/min
Injection volume	:	10 µl
Run time	:	10minutes

Figure no: 5 Chromatogram for trial 4

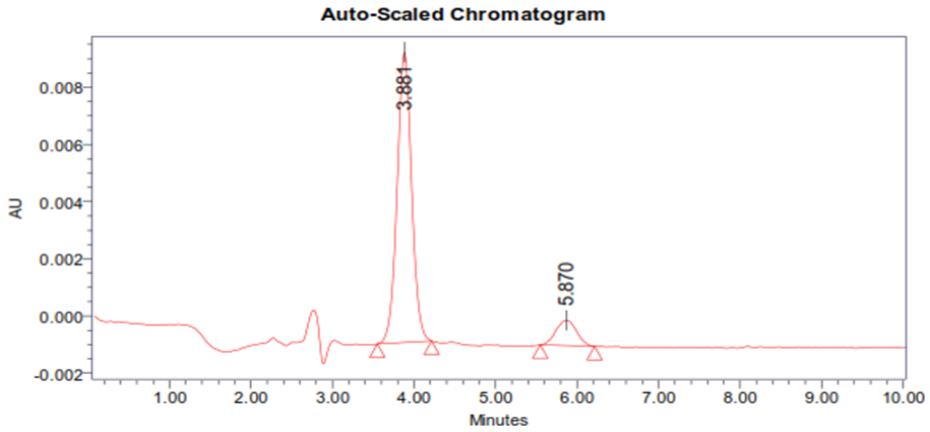


Table no: 12 Peak results for trial 4

S.No.	Peak name	Rt	Area	Height	USP Tailing	USP plate count
1	Trametinib	3.881	465215	32541	0.99	2654

Observation: This trial does not show Proper base line and more tailing in the chromatogram. So go for further trails to obtain good peaks.

Trial 5:

Column	:	Phenomenex Gemini C18 (4.6 x 150mm, 5□m)
Column temperature	:	Ambient
Wavelength	:	246 nm
Mobile phase ratio	:	Methanol: Phosphate Buffer (40:60% v/v)
Flow rate	:	1 mL/min
Auto sampler temperature	:Ambient	
Injection volume	:20 µl	
Run time	: 7.5 minutes	

Figure no:6 Chromatogram for Trial 5

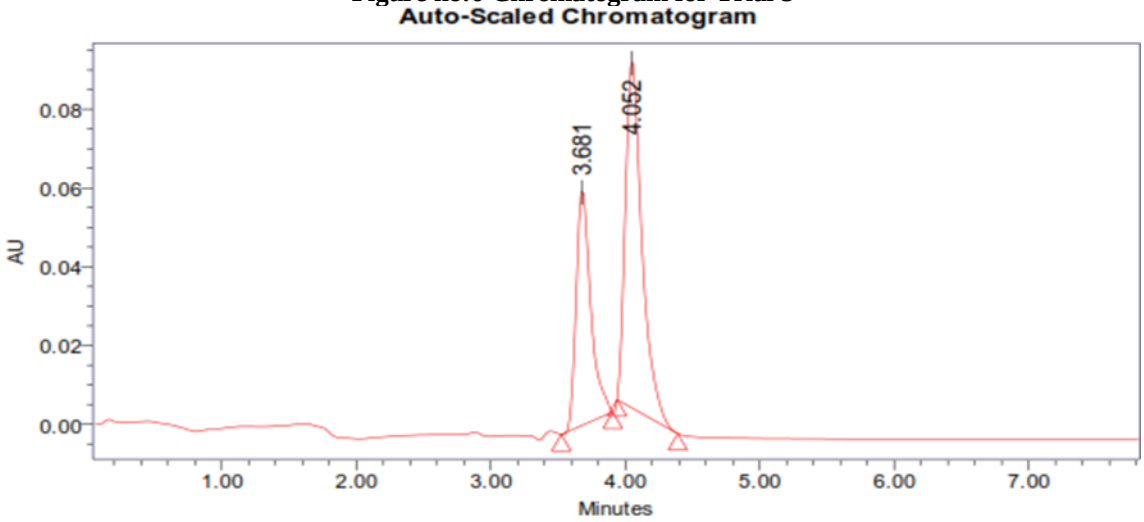


Table no: 13 Peak Results for Trial 5

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Trametinib	3.681	382541	65874	0.94	2698

Observation: This trial shows the improper of chromatogram and baseline. So go for further trails.

Trial 6:

Column : Symmetry Xterra C18 (4.6mm x 150mm, 5□m)
Column temperature : Ambient
Wavelength : 246 nm
Mobile phase ratio : Acetonitrile: Phosphate Buffer (20:80% v/v)
Flow rate : 1mL/min
Auto sampler temperature :Ambient
Injection volume :10 µl
Run time : 7 minutes

Figure no: 7 Chromatogram for Trial 6

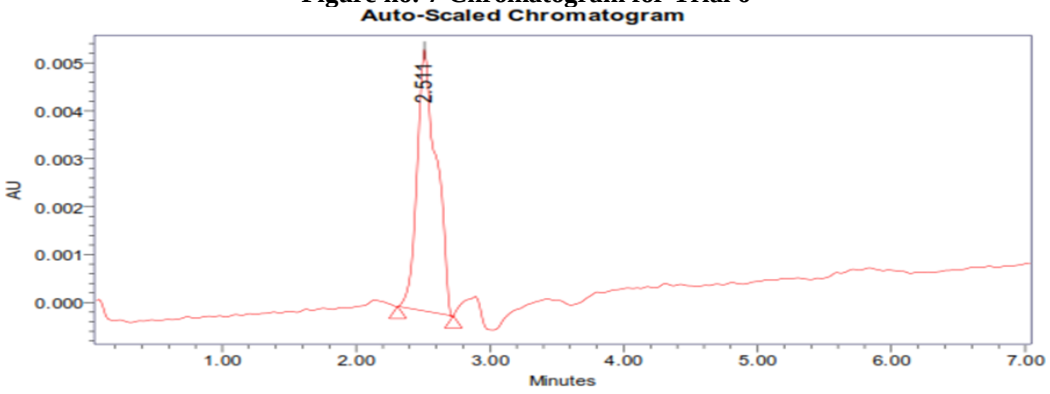


Table no: 14 Peak Results for Trial 6

S.No.	Peak name	Rt	Area	Height	USP Tailing	USP plate count
1	Trametinib	2.511	126545	32546	1.26	986

Observation: In this above trail it shows improper peak shape, baseline and less plate count in the chromatogram. More trails required to get good shape peaks.

Optimized Chromatogram (Standard)

Column : Symmetry Xterra C18 (4.6mm x 150mm, 5□m)
Column temperature : Ambient
Wavelength : 246 nm
Mobile phase ratio : Acetonitrile: Phosphate Buffer (Ph-2.8) (35:65% v/v)Flow
rate : 1.0mL/min
Injection volume : 10 µl
Run time : 8 minutes

Figure no:8 Optimized Chromatogram (Standard)

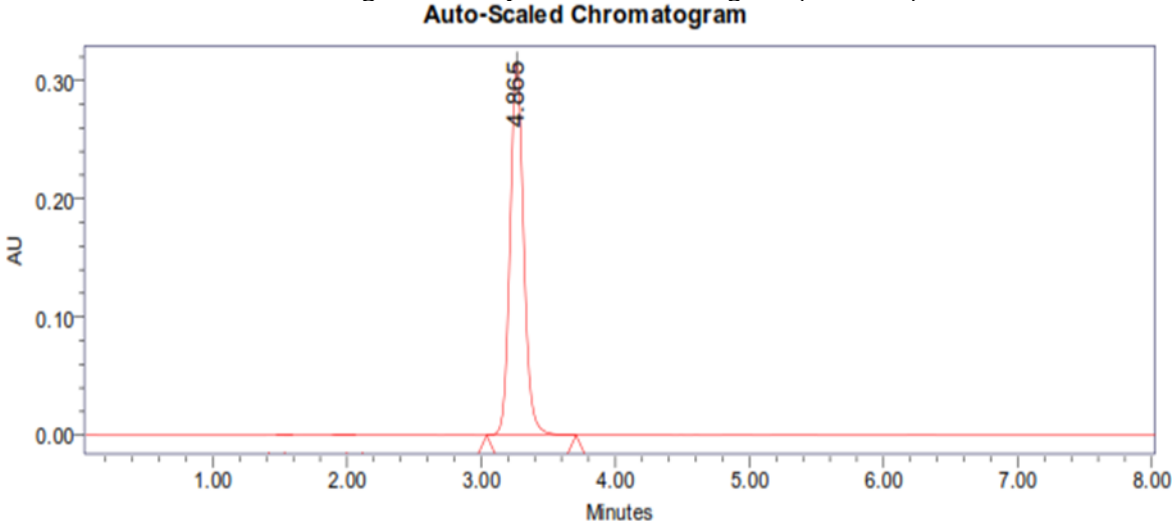


Table no: 15 Peak Results for Optimized Chromatogram (Standard)

S.No.	Peak name	Rt	Area	Height	USP Tailing	USP plate count
1	Trametinib	4.865	856985	69854	1.25	8547

Observation:This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all system suitability parameters. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

Figure no: 9 Optimized Chromatogram (Sample)

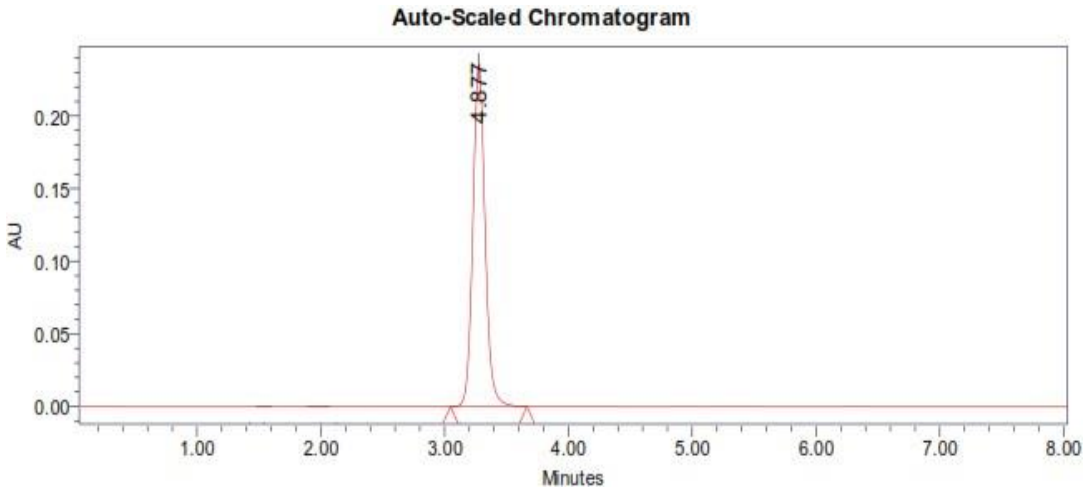


Table no: 16 Results of Optimized Chromatogram (Sample)

S.No.	Name	Retention time (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Trametinib	4.877	865845	69857	1.26	8659

Acceptance criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developedmethod were within the limit.

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 2ml 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 ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer (35:65% v/v) respectively.

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

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

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

PREPARATION OF MOBILE PHASE:

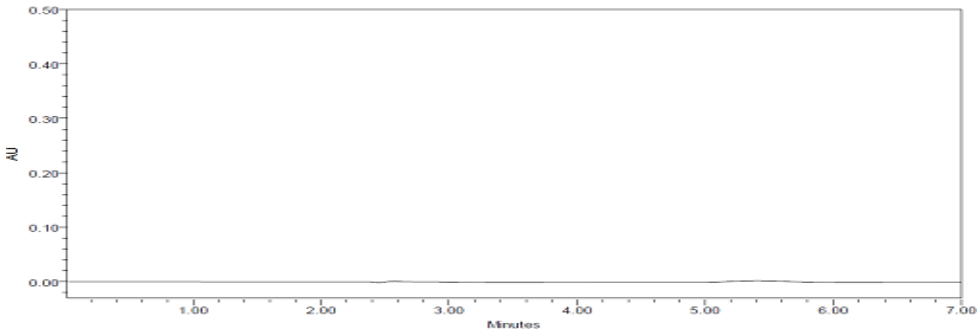
Preparation of mobile phase: Accurately measured 350 ml of Acetonitrile (35%) and 650 ml (65%) Phosphate Buffer were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

METHOD VALIDATION

Figure no: 10 Chromatogram showing blank (mobile phase preparation)

Blank:



SYSTEM SUITABILITY: Accurately weigh and transfer 10 mg of Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 2ml of the above Trametinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: 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.

Figure no: 11 Chromatogram showing injection -1

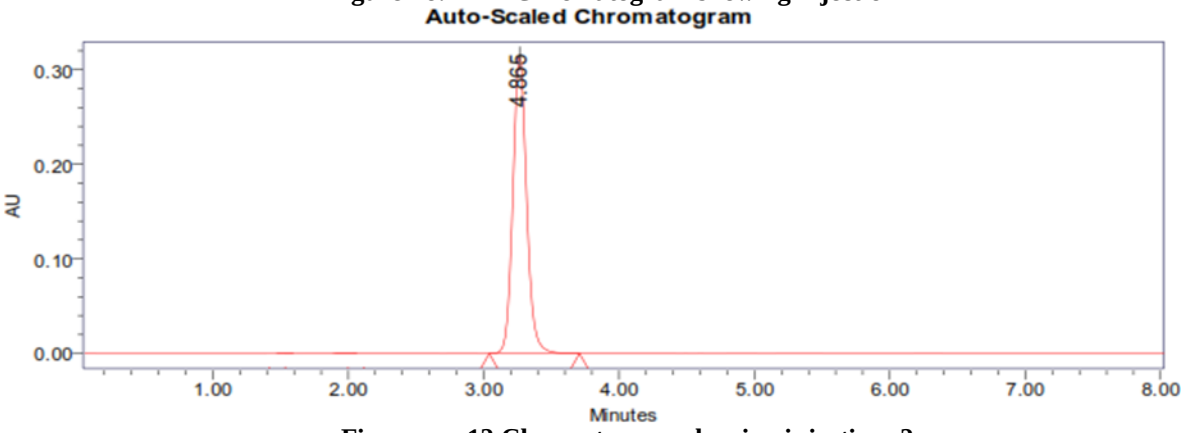


Figure no: 12 Chromatogram showing injection -2

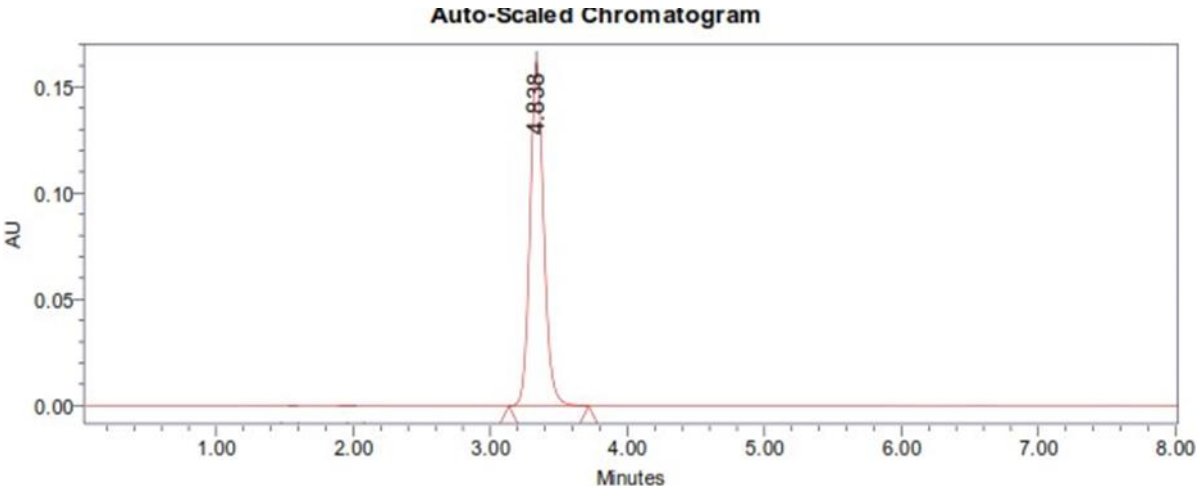


Figure no: 13 Chromatogram showing injection -3

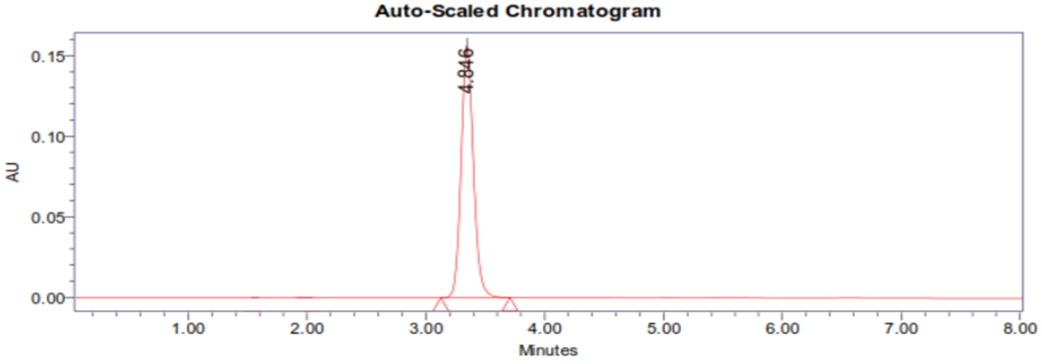


Figure no:14 Chromatogram showing injection -4

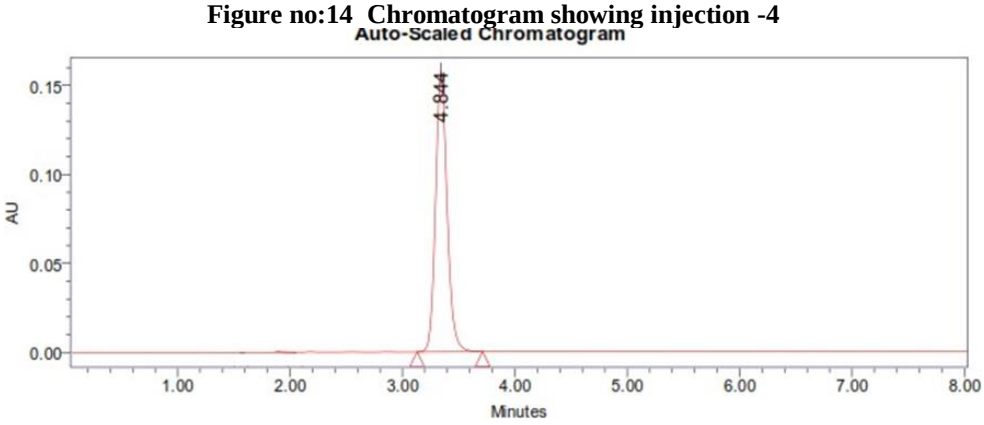


Figure no: 15 Chromatogram showing injection -5

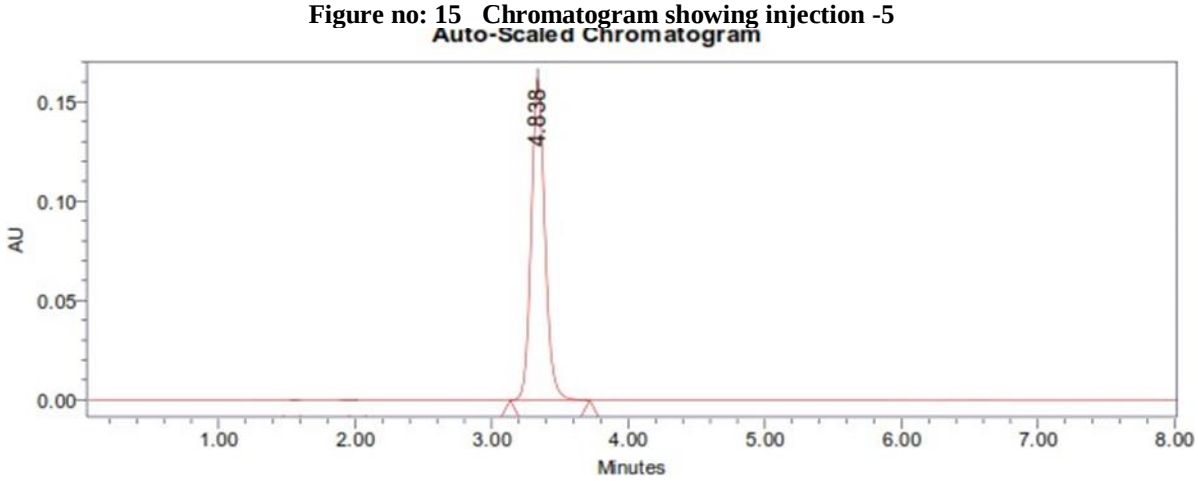


Table no: 17 Results of system suitability for Trametinib

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			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY STUDY OF DRUG:

The ICH documents define specificity as the ability to assess unequivocally the analyte in thepresence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Trametinib in drug product.

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 Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 2ml of the above Trametinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution: Take average weight of the Powder and 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 2ml of the above Trametinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

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

Assay (Standard):

Figure no: 16 Chromatogram showing injection -1

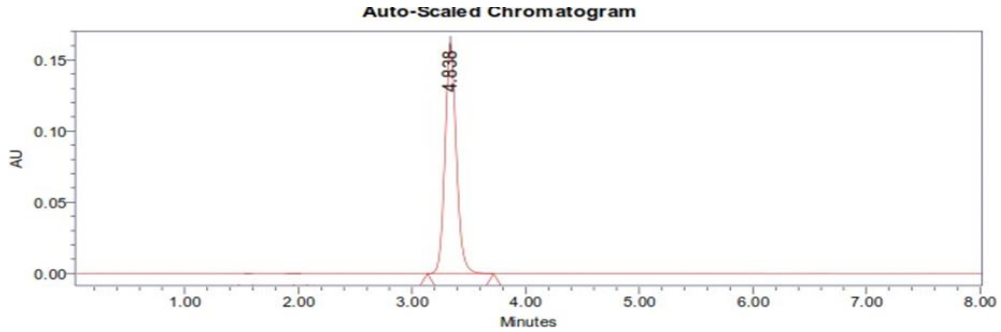


Figure no: 17 Chromatogram showing injection -2

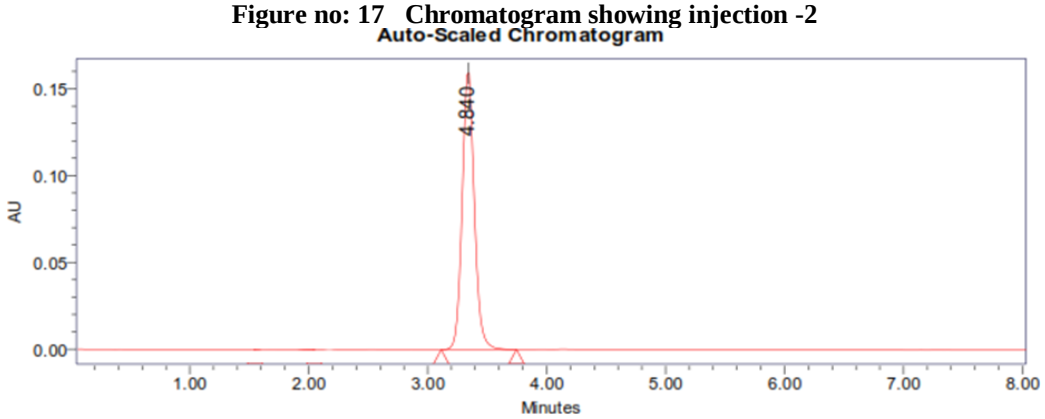


Figure no:18 Chromatogram showing injection -3

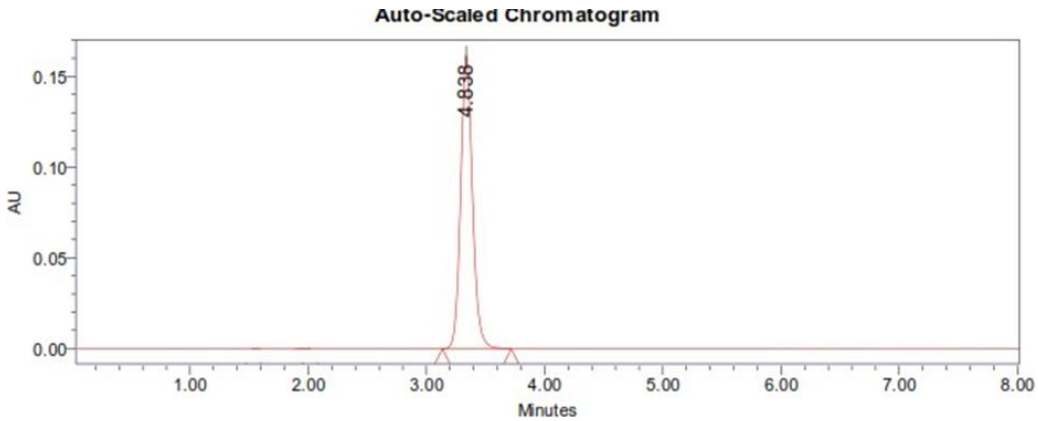


Figure no:19 Chromatogram showing injection -4

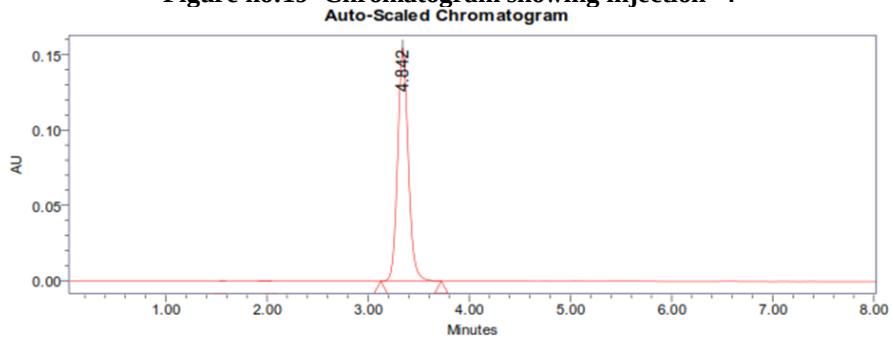


Figure no:20 Chromatogram showing injection -5

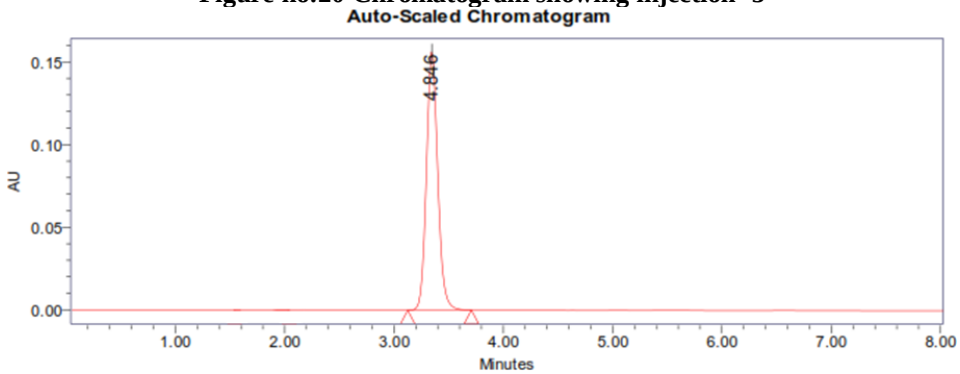


Table no:18 Results of Assay (Standard) for Trametinib

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			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):

Figure no: 21 Chromatogram showing assay of sample injection-1

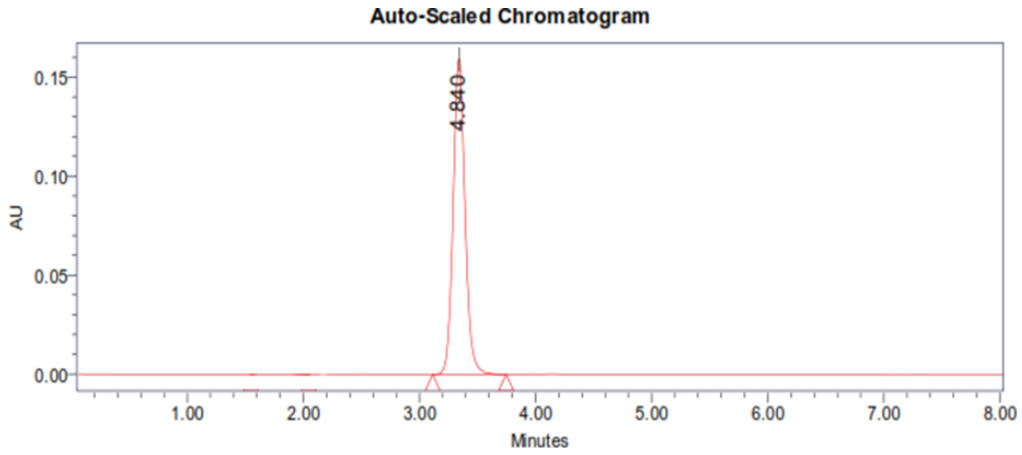


Figure no: 22Chromatogram showing assay of sample injection-2

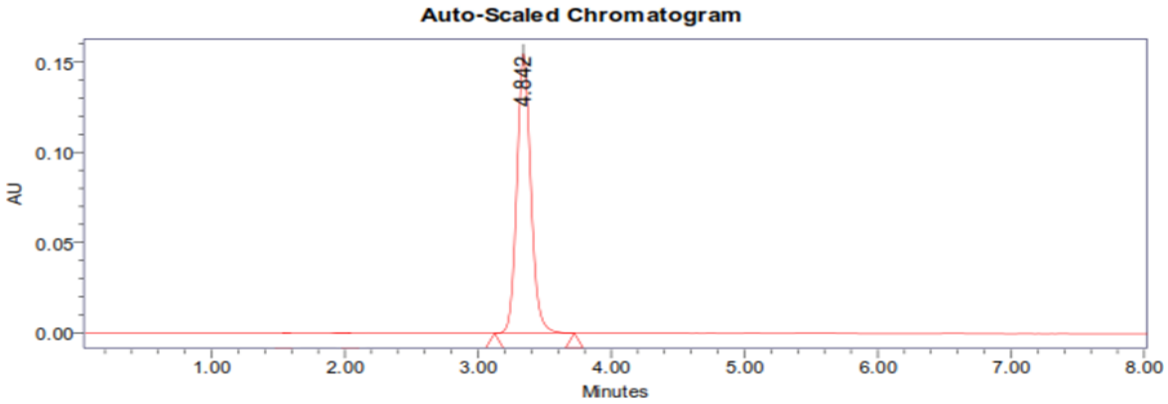


Figure no: 23 Chromatogram showing assay of sample injection-3

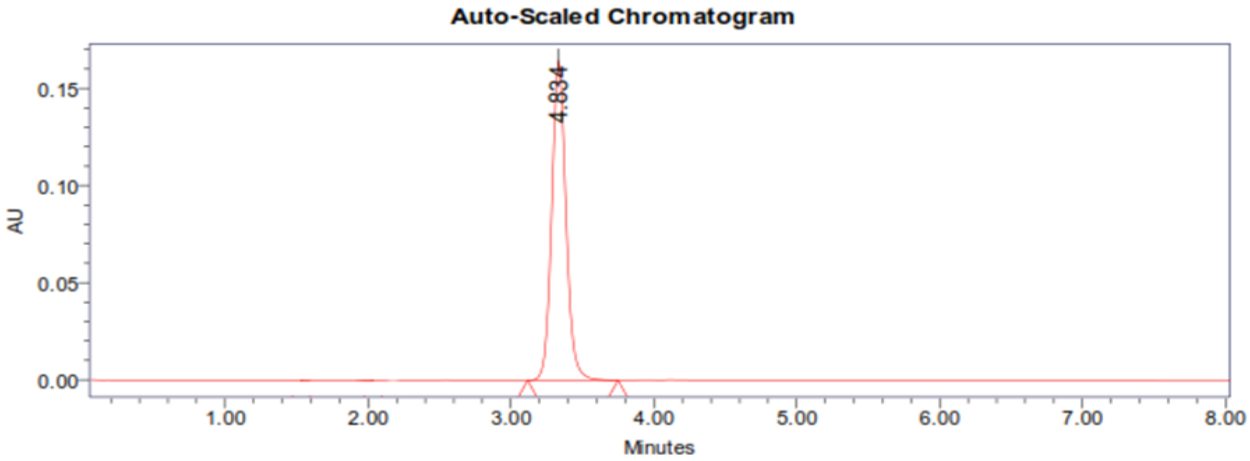


Table no: 19 Peak results for Assay sample

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

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

The % purity of Trametinib in pharmaceutical dosage form was found to be 99.87%.

LINEARITY

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY: Accurately weigh and transfer 10 mg of Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (10ppm of Trametinib):

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up tomark with diluent.

Preparation of Level – II (15ppm of Trametinib): Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up tomark with diluent.

Preparation of Level – III (20ppm of Trametinib): Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up tomark with diluent.

Preparation of Level – IV (25ppm of Trametinib): Take 0.25ml of stock solution in to 10ml of volumetric flask and make up the volume up tomark with diluent.

Preparation of Level – V (30ppm of Trametinib): Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up tomark 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 Peakarea) and calculate the correlation coefficient.

Figure no: 24 Chromatogram showing linearity level-1

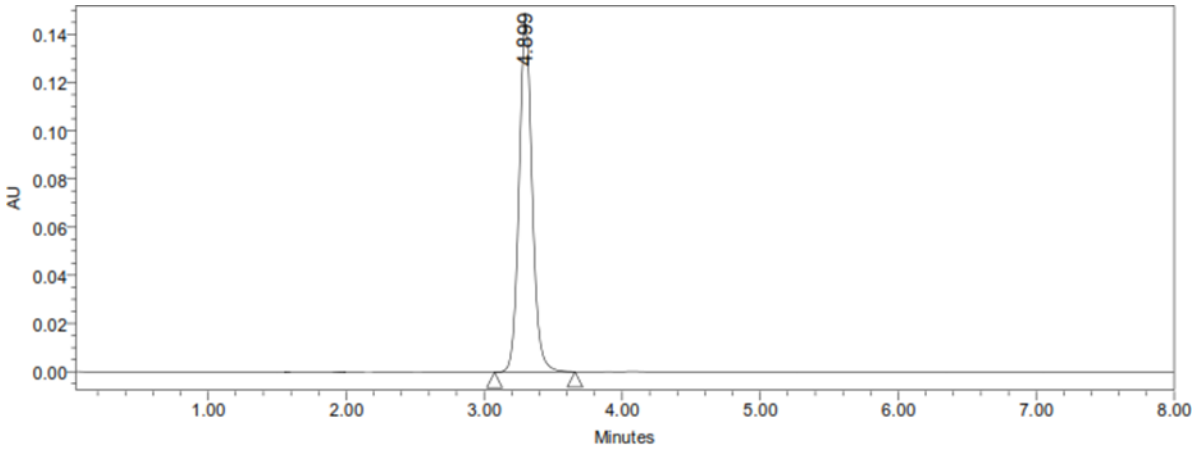


Figure no: 25 Chromatogram showing linearity level-2

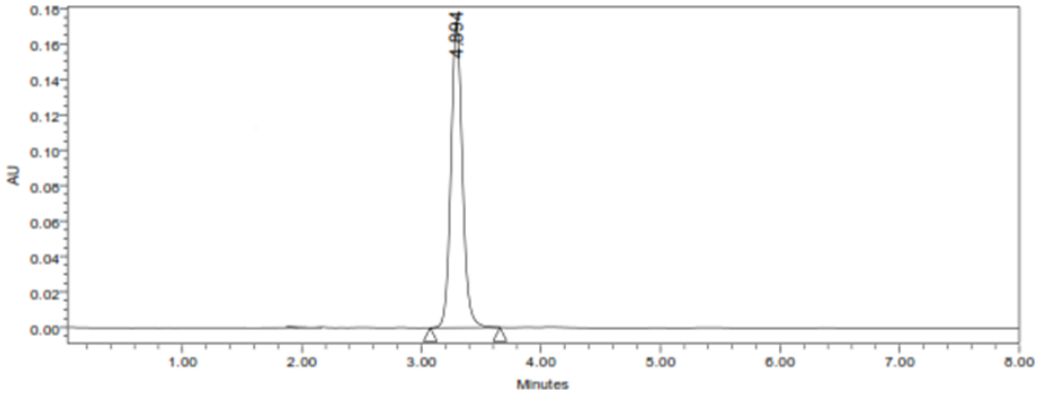


Figure no: 26 Chromatogram showing linearity level-3

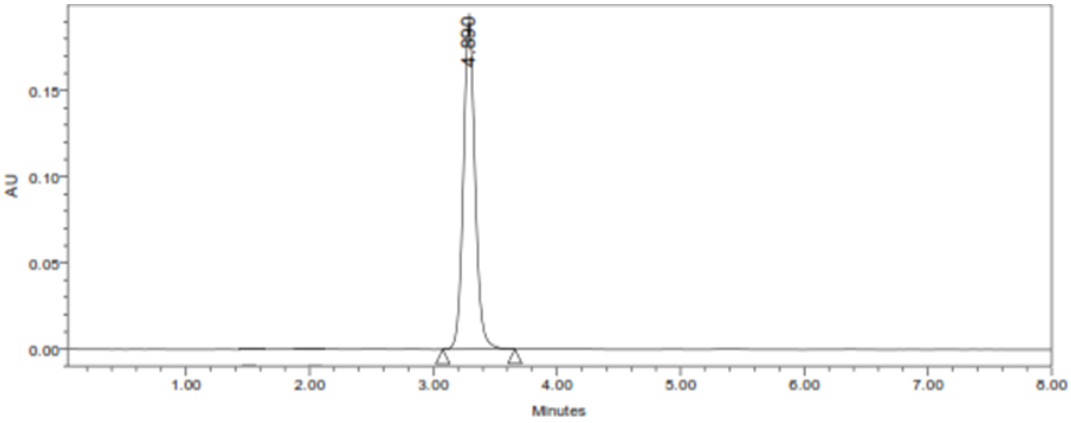


Figure no: 27 Chromatogram showing linearity level-4

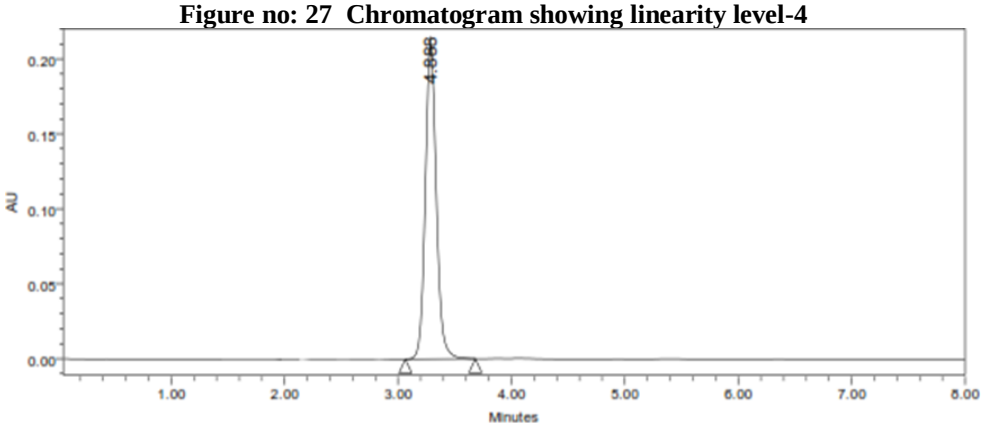
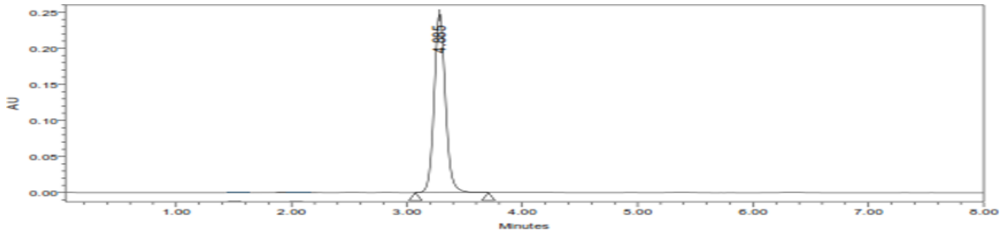


Figure no: 28 Chromatogram showing linearity level-5

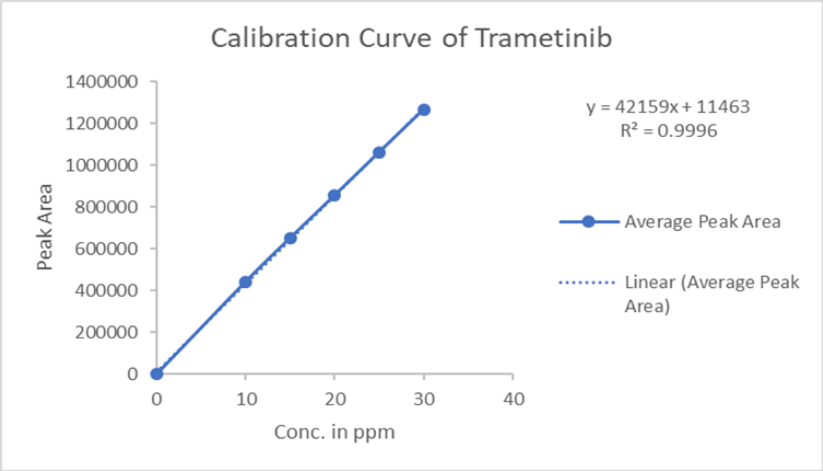


CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Table no: 20 Data for Linearity of Trametinib

Concentration (g/ml)	Average Peak Area
10	442986
15	652547
20	856985
25	1063654
30	1268475

Figure no: 29 calibration curve of Trametinib



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Trametinib is a straight line.

Y = mx + c

Slope (m) = 42159 Intercept
(c) = 11463
Correlation Coefficient (r) = 0.99

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 11463. These values meet the validation criteria.

PRECISION: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Preparation of Trametinib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Take 2ml of stock solution in to 10ml of volumetric flask and make up the volume up to 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 specifiedlimits. Obtained Five (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Figure no: 30 Chromatogram showing precision injection -1

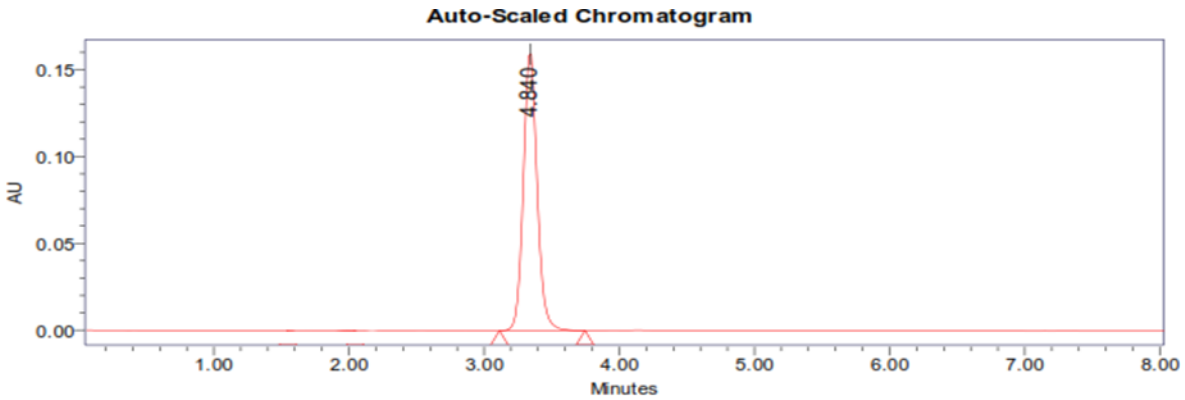


Figure no: 31 Chromatogram showing precision injection -2

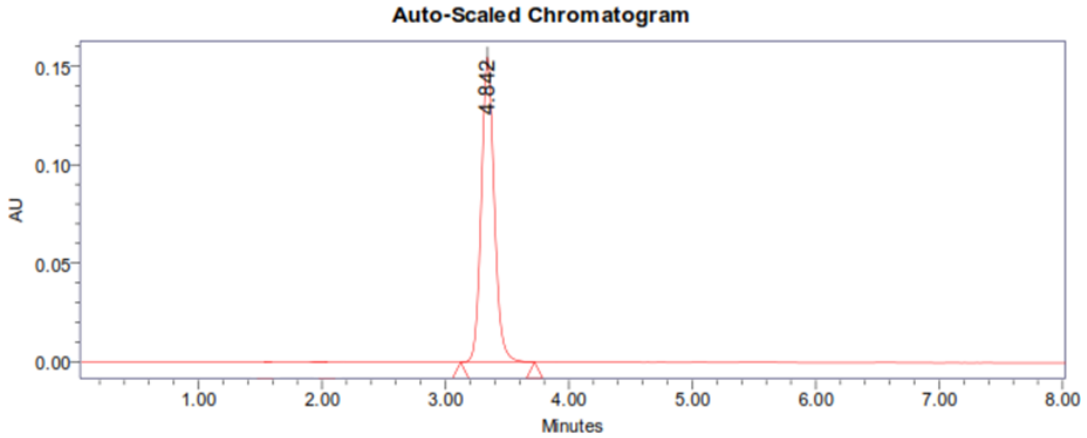


Figure no: 32 Chromatogram showing precision injection -3

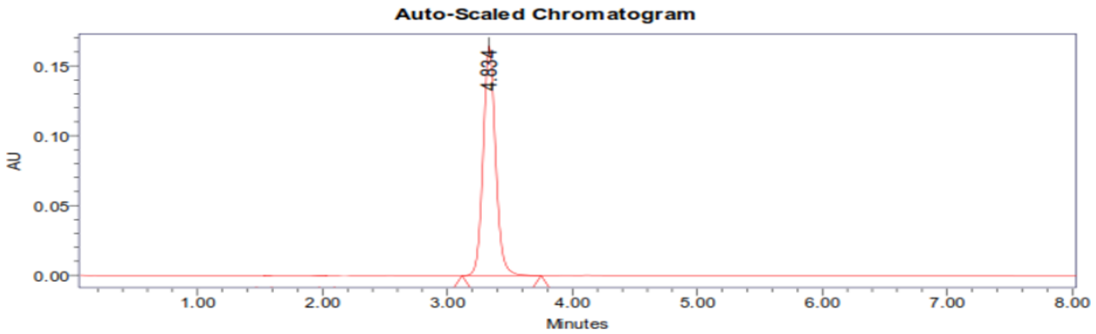


Figure no:33 Chromatogram showing precision injection -4

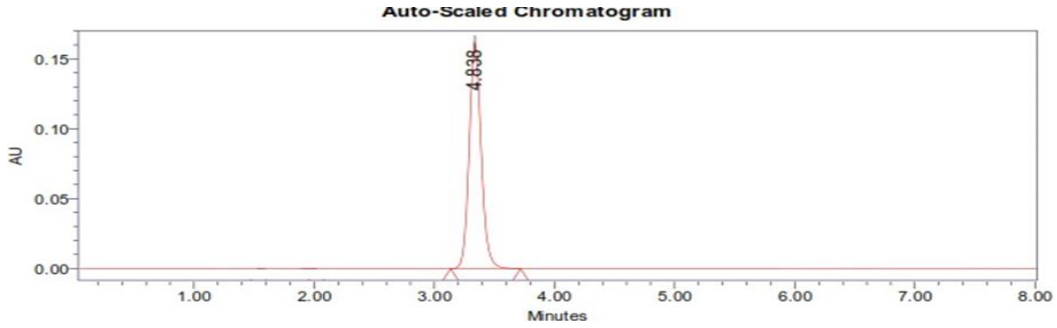


Figure no: 34 Chromatogram showing precision injection -5

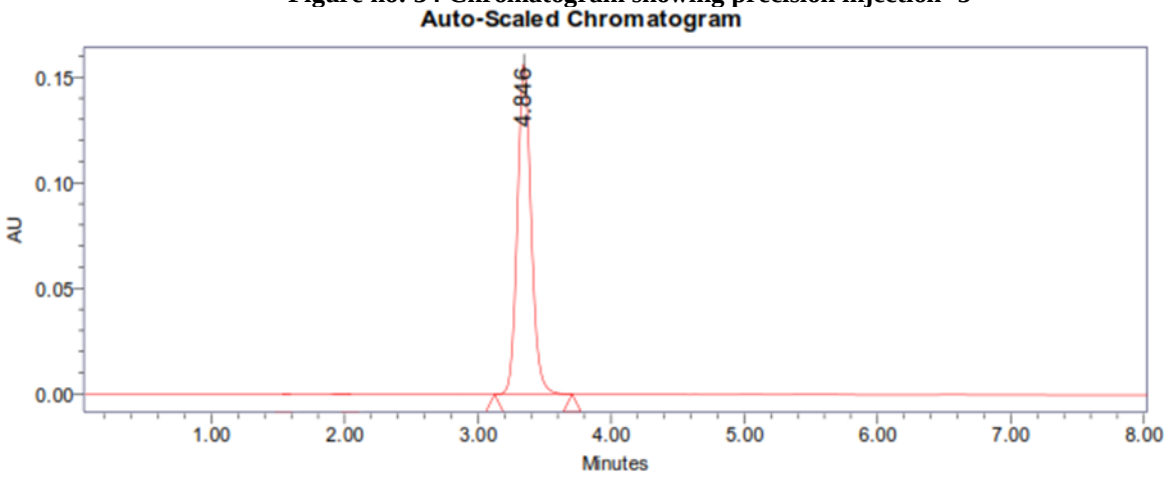


Figure no: 35 Chromatogram showing precision injection -6

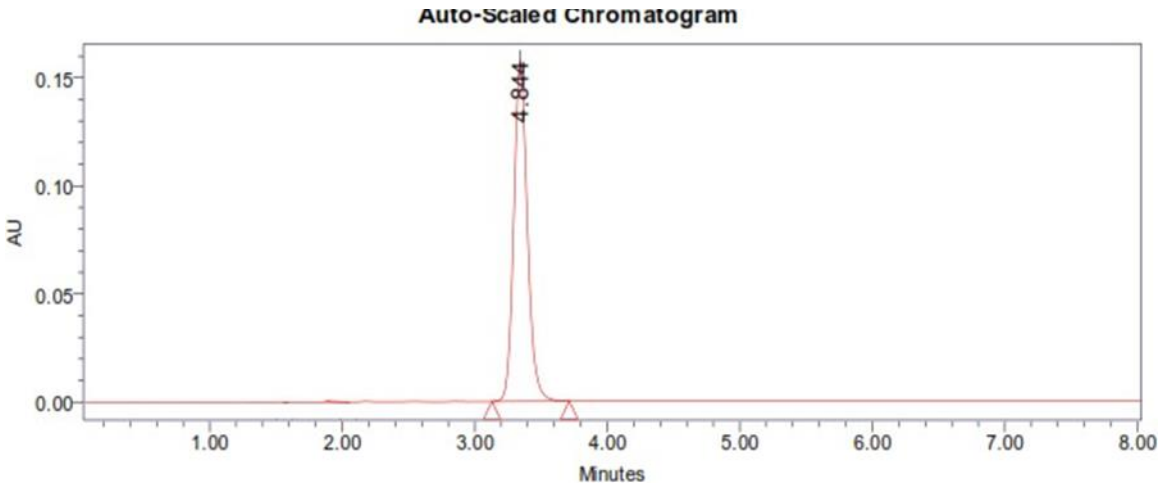


Table no:21 Results of method precision for Trametinib:

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			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hencemethod is precise.

Intermediate precision:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precisionwas performed on different days by maintaining same conditions.

Procedure:
Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within thespecified limits.

Figure no: 36 Chromatogram showing Analyst 1 injection -1

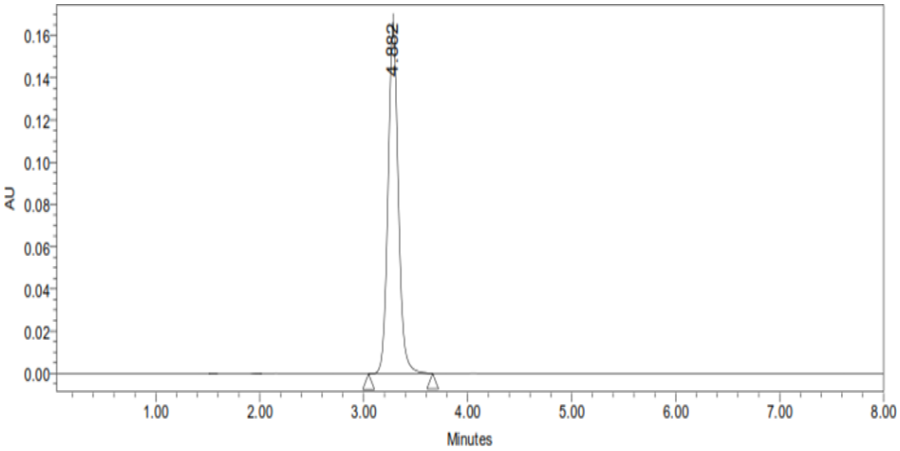


Figure no: 37 Chromatogram showing Analyst 1 injection -2

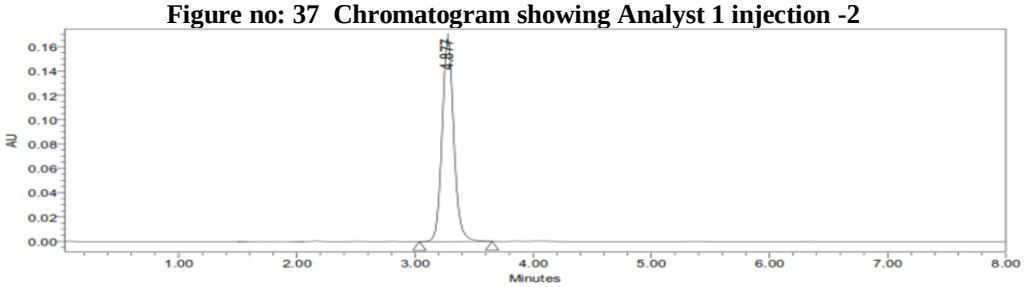


Figure no: 38 Chromatogram showing Analyst 1 injection -3

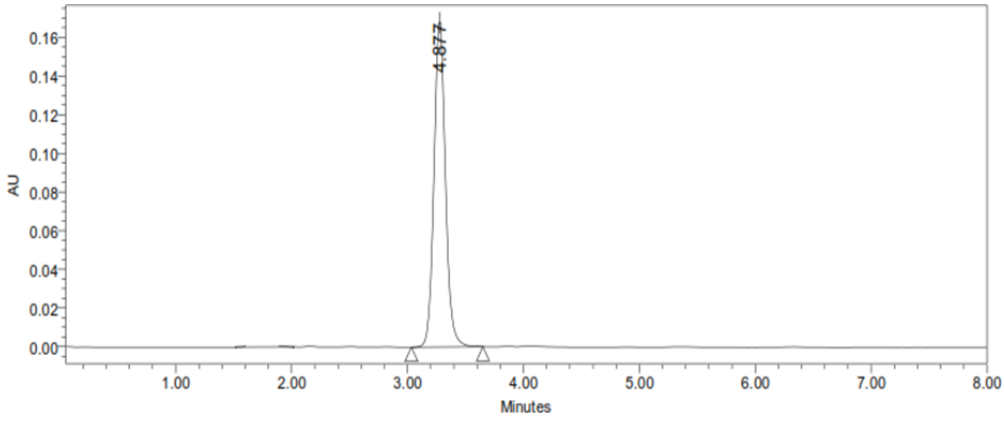


Figure no: 39 Chromatogram showing Analyst 1 injection -4

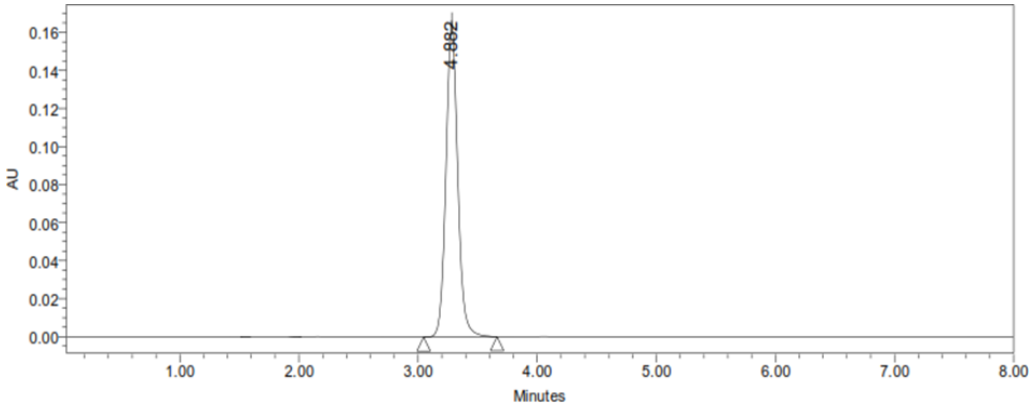


Figure no: 40 Chromatogram showing Analyst 1 injection -5

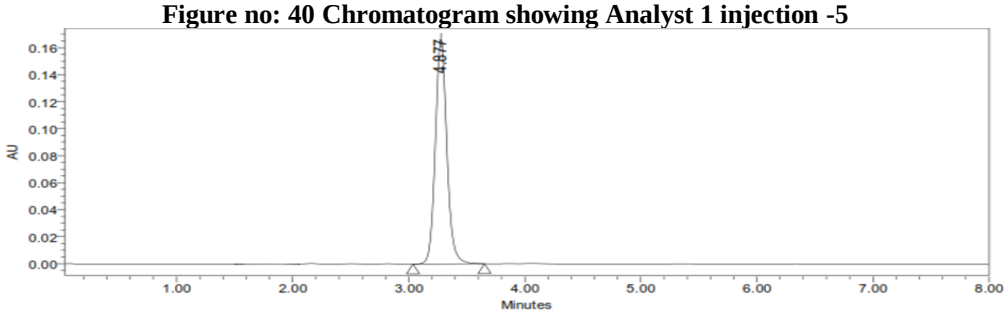


Figure no: 41 Chromatogram showing Analyst 1 injection -6

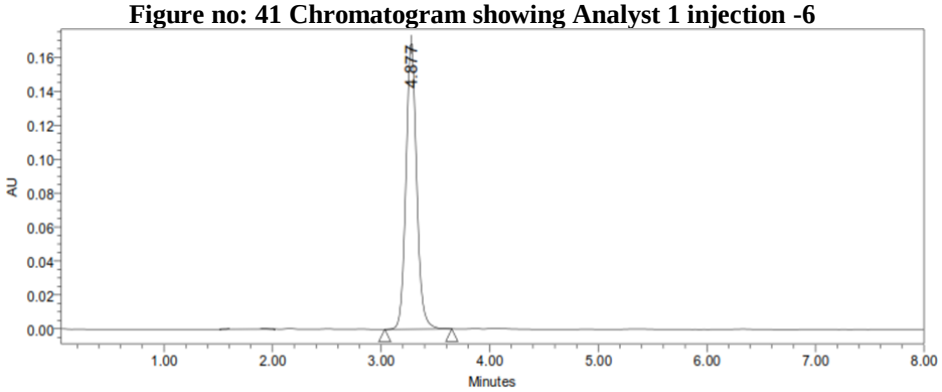


Table no:22 Results of ruggedness for Trametinib

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			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections inHPLC. The %RSD for the area of six replicate injections was found to be within the specifiedlimits.

Figure no: 42 Chromatogram showing Analyst 2 injection -1

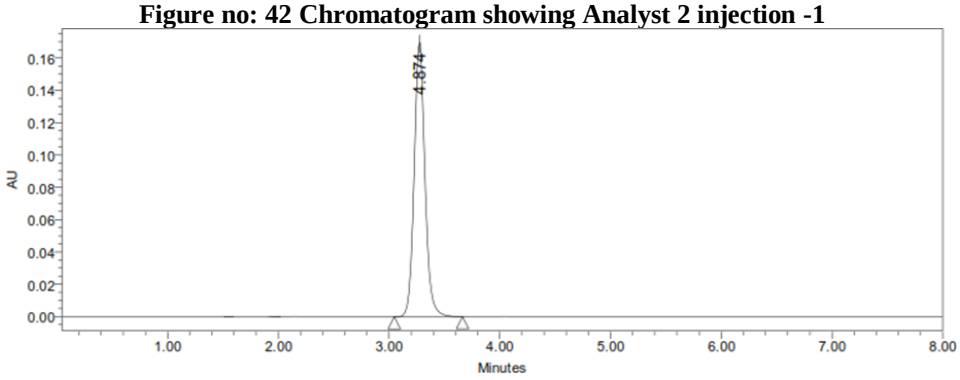


Figure no: 43 Chromatogram showing Analyst 2 injection -2

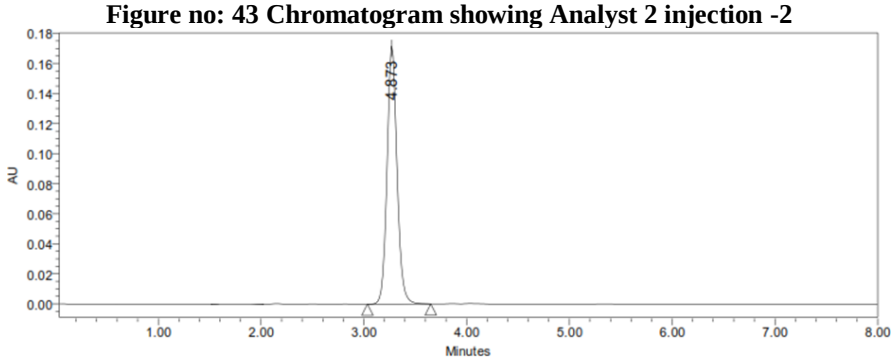


Figure no: 44 Chromatogram showing Analyst 2 injection -3

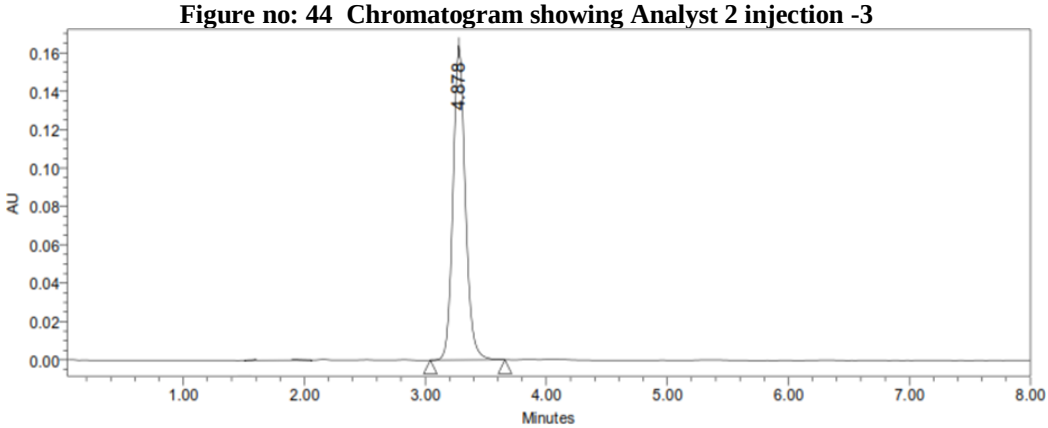


Figure no: 45 Chromatogram showing Analyst 2 injection -4

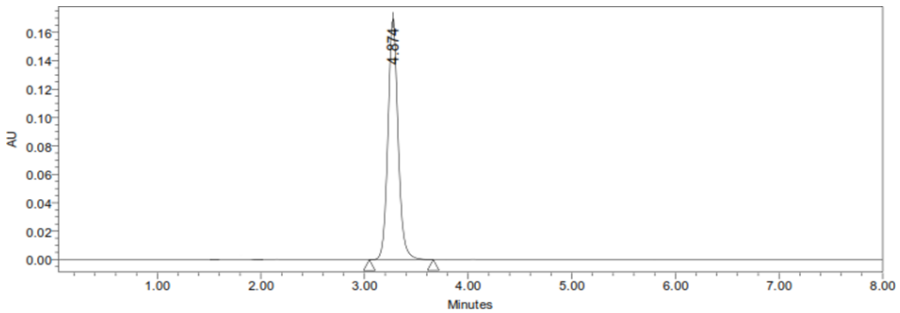


Figure no: 46 Chromatogram showing Analyst 2 injection -5

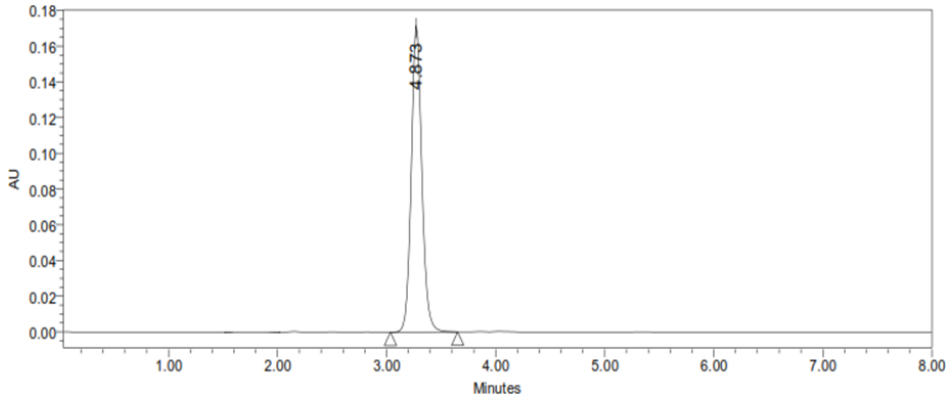


Figure no: 47 Chromatogram showing Analyst 2 injection -6

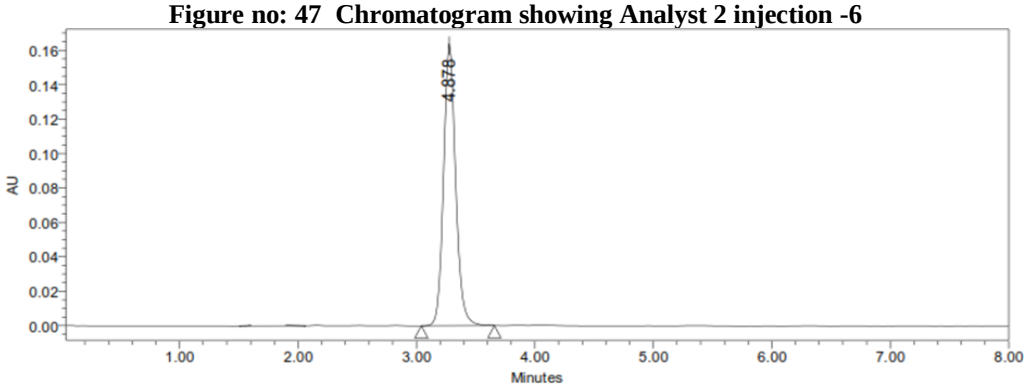


Table no:23 Results of Intermediate precision Analyst 2 for Trametinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trametinib	4.874	878548	70254	8758	1.26
2	Trametinib	4.873	874598	70265	8798	1.27
3	Trametinib	4.878	874589	69989	8742	1.26
4	Trametinib	4.874	875984	70145	8759	1.26
5	Trametinib	4.873	875981	70158	8746	1.27
6	Trametinib	4.878	875984	69998	8796	1.27
Mean			875947.3			
Std. Dev.			1444.511			
% RSD			0.164908			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

ACCURACY:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Take 1ml of stock solution in to 10ml of volumetric flask and make up the volume up to markwith diluent.

For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution).
Take 2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
For preparation of 150% Standard stock solution: Accurately weigh and transfer 10 mg of Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution)**.**Take 3ml of stock solution in to 10ml of volumetric flask and make up the volume up to 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.

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Accuracy 50%:

Figure no: 48 Chromatogram showing accuracy-50% injection-1

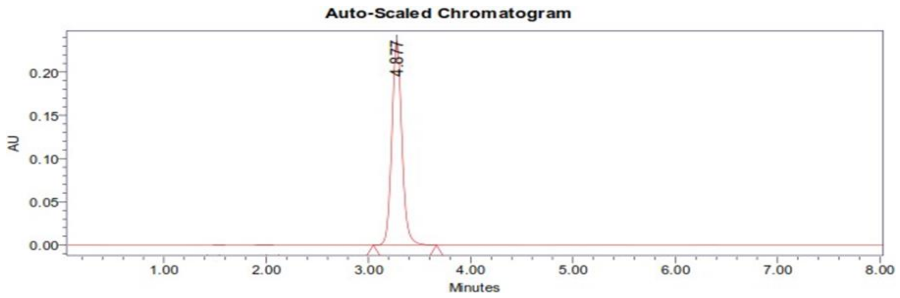


Figure no: 49 Chromatogram showing accuracy-50% injection-2

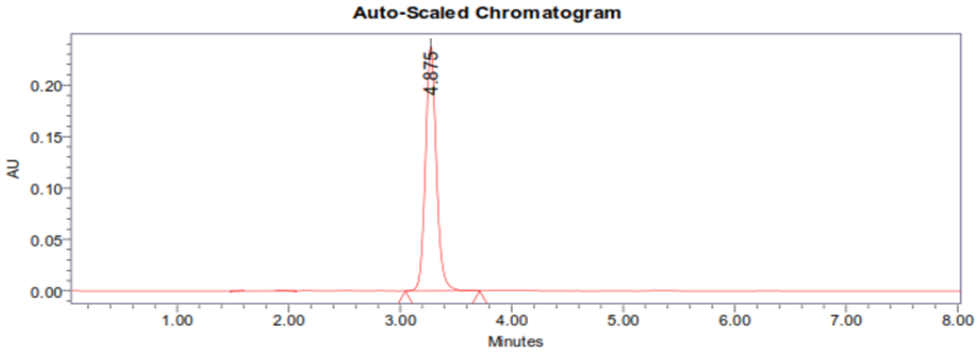


Figure no: 50 Chromatogram showing accuracy-50% injection-3

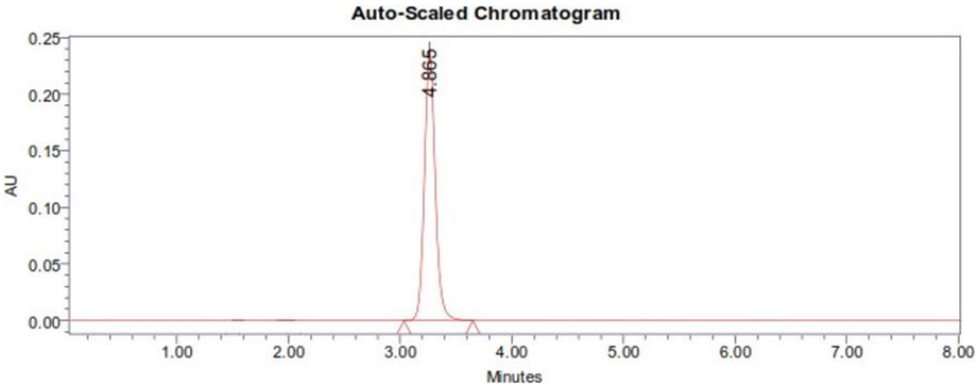


Table no:24 Results of Accuracy for sample concentration-50%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trametinib	4.877	429795	39865	1.15	4985	1
2	Trametinib	4.875	428989	39685	1.16	4974	2
3	Trametinib	4.865	429865	39587	1.15	4952	3

Accuracy 100%:

Figure no: 51 Chromatogram showing accuracy-100% injection-1

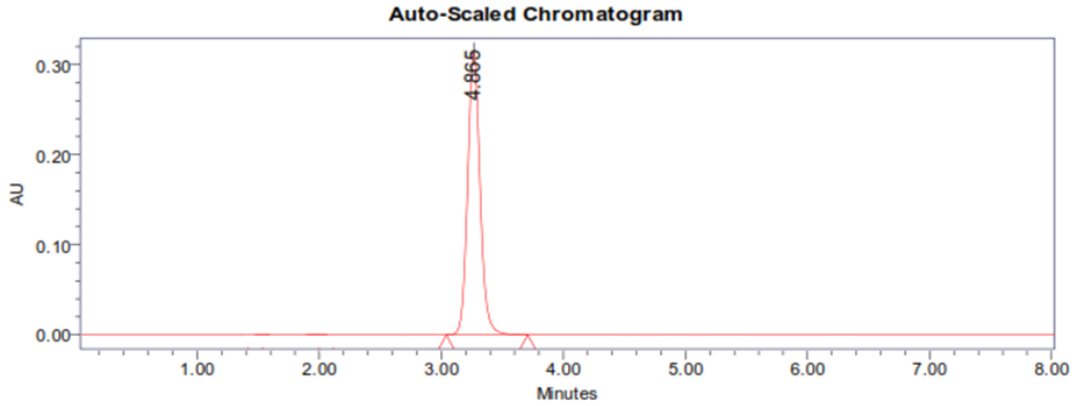


Figure no: 52 Chromatogram showing accuracy-100% injection-2

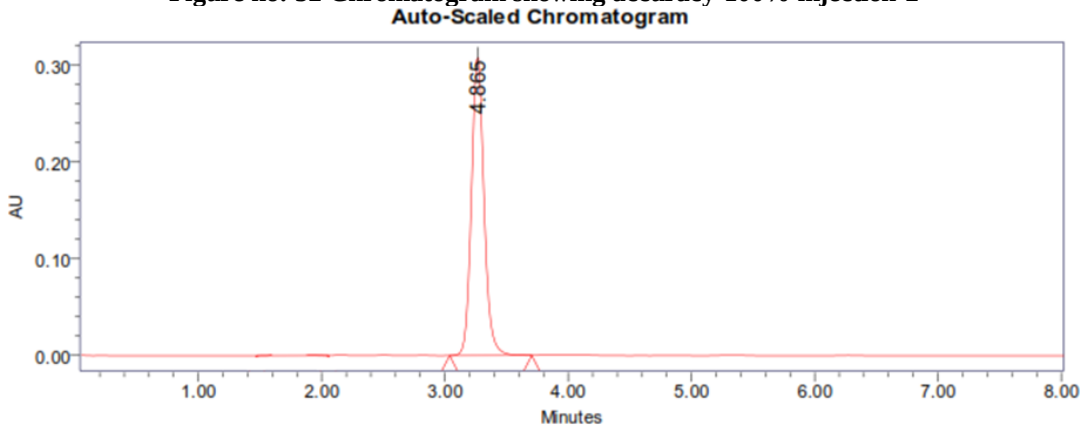


Figure no: 53 Chromatogram showing accuracy-100% injection-3

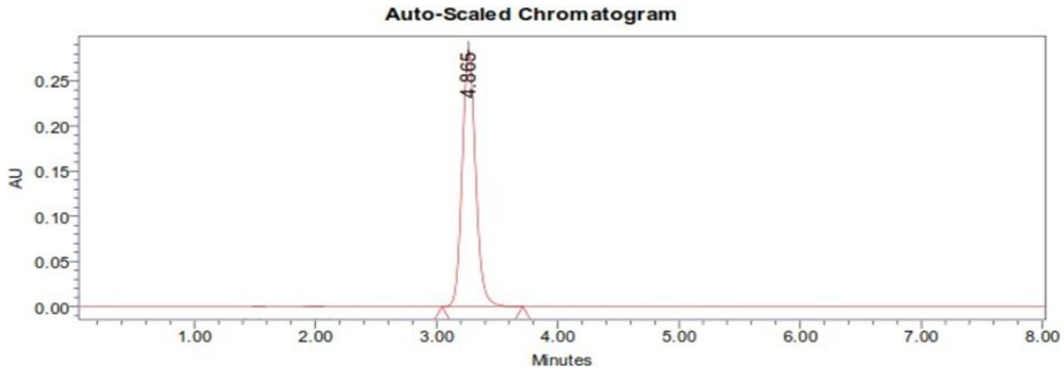


Table no:25 Results of Accuracy for concentration-100%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trametinib	4.865	856963	865985	1.29	8659	1
2	Trametinib	4.865	856748	867458	1.28	8657	2
3	Trametinib	4.865	854857	865987	1.28	8695	3

Accuracy 150%:

Figure no: 54 Chromatogram showing accuracy-150% injection-1

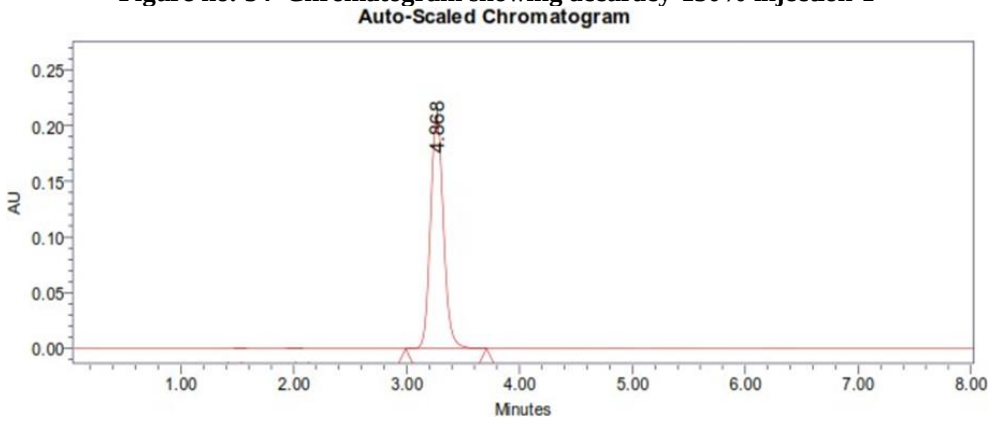


Figure no: 55 Chromatogram showing accuracy-150% injection-2

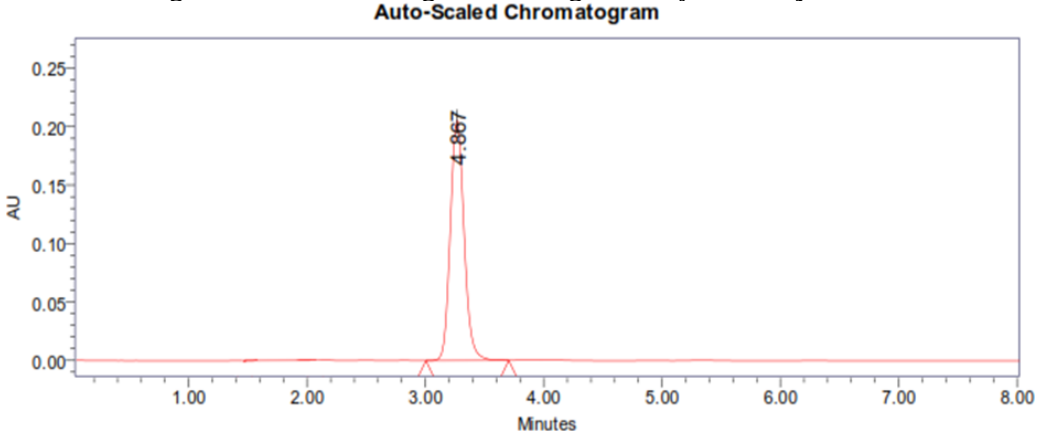


Figure no: 56 Chromatogram showing accuracy-150% injection-3

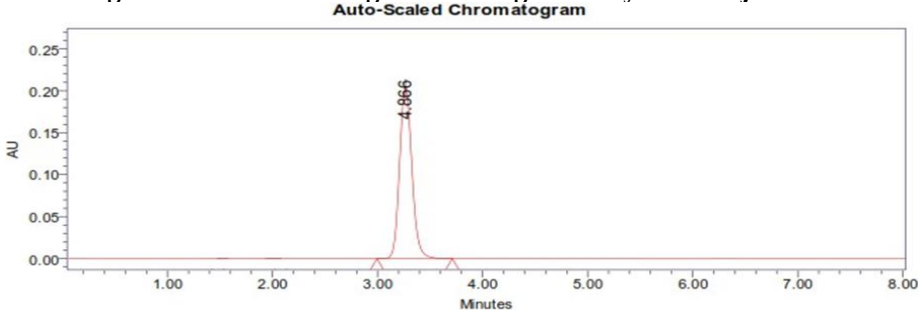


Table no: 26 Results of Accuracy for concentration-150%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trametinib	4.868	1279562	97885	1.85	9895	1
2	Trametinib	4.867	1268542	98569	1.86	9867	2
3	Trametinib	4.866	1269498	98798	1.85	9849	3

Table no: 27 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%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method isaccurate.

LIMIT OF DETECTION FOR TRAMETINIB

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

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response
S = Slope of the calibration curve
Result:= 1.3µg/ml

LIMIT OF QUANTITATION FOR TRAMETINIB

The quantitation limit of an individual analytical procedure is the lowest amount ofanalyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where
σ = Standard deviation of the response
S = Slope of the calibration curve
Result:= 3.9µg/ml

ROBUSTNESS: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Trametinib. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Trametinib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For 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 Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Take 2ml of stock solution in to 10ml of volumetric flask and make up the volume up to markwith diluent.

Effect of Variation of flow conditions: The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl 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 40:60, 30:70 instead of 35:65, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Variation in flow

Figure no: 57 Chromatogram showing less flow of 0.9ml/min

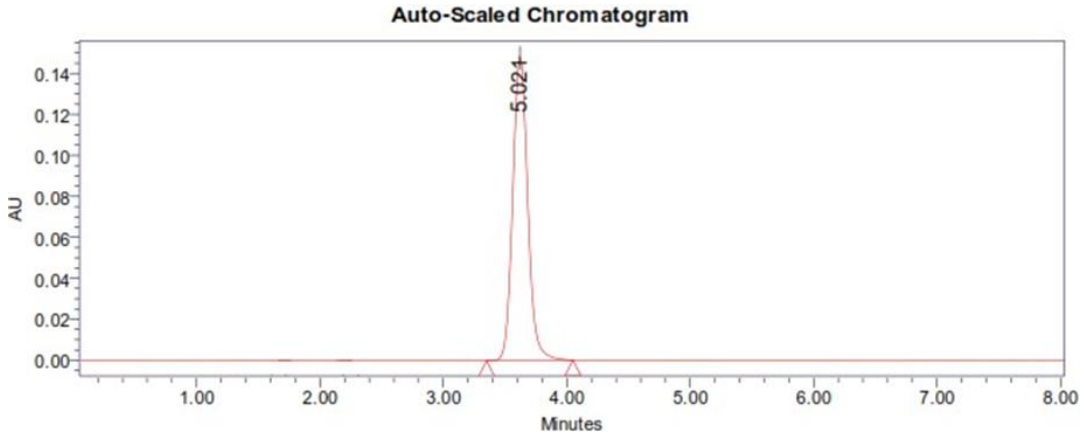
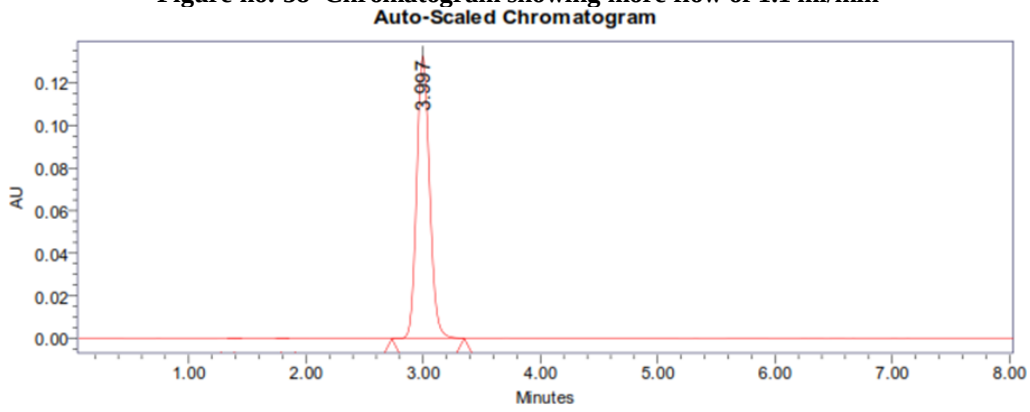


Figure no: 58 Chromatogram showing more flow of 1.1 ml/min



Variation of mobile phase organic composition

Figure no: 59 Chromatogram showing less organic composition

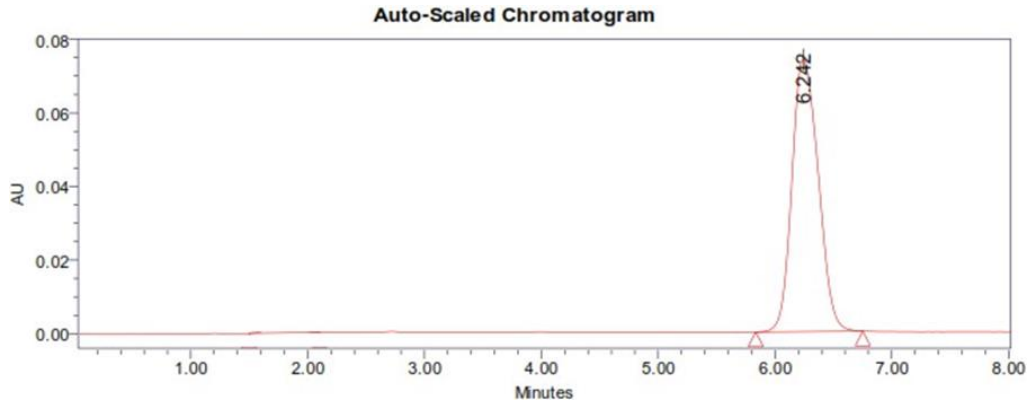


Figure no: 60 Chromatogram showing more organic composition

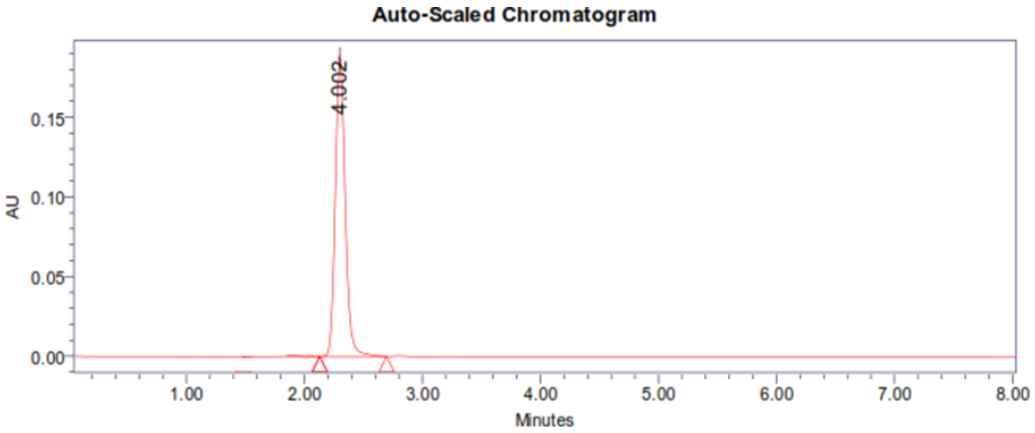


Table no: 28 Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
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

Acceptance criteria: The tailing factor should be less than 2.0 and the number of theoretical plates (N) should bemore than 2000.

Summary

The solubility of the drug was determined. The scanning of drugs for wavelength in UV region was carried out and wavelength was selected by using photo-diode array detector for the measurement of active ingredients in the proposed method. In HPLC method, the conditions were optimized to obtain an adequate elution of compounds. Initially, various mobile phase compositions were tried to separate the titled ingredients. Mobile phase, column selection, wavelength selection was based on peak parameters (height, tailing factor, theoretical plates, capacity or symmetry factor) and run time. The mobile phase with acetonitrile: phosphate buffer (35:65 % v/v) in isocratic program and a flow rate of 1ml/min was used. The optimum wavelength for detection was 246 nm and a run time of 8 min. the HPLC method used for the estimation of trametinib from tablets was validated in terms of system suitability, accuracy, precision, linearity, filter validation, solution stability, robustness and ruggedness.

Table no: 29 Summary of Results and Discussion

Validation Parameter	Acceptance Criteria	HPLC Results
System Suitability	The RSD should be NMT 2% for 5 replicate injections for each peak, theoretical plates NLT 2000,Tailing factor NMT 2, Resolution NLT 2	The system suitability parameters like resolution, Tailing factor, theoretical plates all complies as per the specification
Accuracy	The % Recovery at each spike level shall be NLT 98.0.0% and NMT 102.0.0% of the added amount.	99.68
System Precision	The % RSD of peaks obtained from the 6 replicate injections should be NMT 2.0%	0.188
Intermediate Precision (ID 1 and ID 2)	The %RSD for the six determinations shall be NMT 2.0%	0.118 and 0.164
Linearity	The correlation coefficient shall be NLT 0.999	0.999
Robustness	All system suitability parameters should pass for all conditions.	The system suitability parameters passed for all the conditions
Ruggedness	All the system suitability parameters should pass for all the conditions.	The system suitability parameters passed for all the conditions
Specificity	No interference of blank and peak purity should pass in all the conditions	No interference was observed and passed the peak purity in all conditions (99.87%)

CONCLUSION:

With the proposed method developed & validation, it was found to be rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and economical. The method shows non-interference of formulation excipients in the estimation with advantage of drug retention time below 5 min & the drug can be assayed with in short time. Thus, the method is less time consuming & can be used in laboratories for the routine analysis of single & combination of drugs.

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