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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TEPOTINIB IN PRESENCE OF ITS IMPURITIES IN A TABLET DOSAGE FORM

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Keywords:

RP-HPLC, Tepotinib, Impurity-1&2, tablet dosage form, Forced degradation studies, Acetonitrile, KH_2PO_4

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ABSTRACT: A simple, rapid, precise, sensitive and reproducible Reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Tepotinib in presence of its impurities (1&2) in pharmaceutical dosage form. Chromatographic separation of Tepotinib and its known impurities were achieved on Waters Allianace-e 2695 by using Inertsil ODS C18 250x4.6mm 5µ column and the mobile phase containing (0.01M) KH_2PO_4 PH-2.5&Acetonitrile in the ratio of 50:50% v/v. The flow rate was 1.0ml/min; detection was carried at 263nm using a photodiode array detector at ambient temperature. The calibration range was 10-60µg/ml for Tepotinib, 2.5-15µg/ml for Imp-1& 3.75-22.5µg/ml for Imp-2. %Relative standard deviation of peak areas of all the measurements less than 2%. Recovery was obtained between 98-102%. Stability studies were also performed with known impurities the drug showed maximum degradation in alkaline stress condition. The proposed method was validated according to ICH guidelines. The results obtained were within the acceptable limits. The proposed method was found to be simple, economical, precise, accurate & robust for quantitative analysis of Tepotinib in presence of its impurities.

INTRODUCTION: Tepotinib[1] is chemically known as 3-[1-[[3-[5-[(1-methylpiperidin-4-yl) methoxy] pyrimidin- 2-yl] phenyl] methyl]-6-oxopyridazin-3-yl] benzonitrile with molecular formula: $C_{29}H_{28}N_6O_2$. Impurity-1 chemically known as 3-(1, 6-dihydro-6-oxopyridazin-3-yl) benzonitrile.Impurity-2 chemically known as2-(3-ethylphenyl) pyrimidin-5-ol. Mesenchymal-epithelial transition factor (MET) [2] is a receptor tyrosine kinase found over expressed and mutated in a variety of tumour types, thus making it a desirable target in their treatment. Tepotinib is a kinase inhibitor directed against MET, including variants with axon 14 skipping it inhibits MET phosphorylation and subsequent downstream signalling pathways in order to inhibit tumour cell proliferation, anchorage independent growth and migration of MET dependent tumour cells. Tepotinib has also been observed to down regulate the expression of epithelial- mesenchymal transition (EMT) promoting genes (e.g. MMP7, COX-2, WNT1, MUC5B and c- MYC) and upregulate the expression of EMT –suppressing genes (e.g. MUC5AC, MUC6, GSK3β and E-cadherin) in c-MET-induced EMT.

It has also been shown to inhibit melatonin 1B and nischarin at clinically relevant concentrations, though the relevance of this activity in regards to Tepotinib mechanism of action is unclear. The survey of literature revealed that there was few HPLC analytical methods [3, 4, 5, 6, 7] has been reported and there was no stability indicating validated analytical methods. Hence authors made

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an attempt to develop validated stability indicating RP-HPLC method for the estimation of Tepotinib in presence of its impurities. The aim of the proposed work to develop accurate, sensitive, precise and economical validated stability indicating RP-HPLC method for the estimation of Tepotinib in presence of its known impurities in bulk and its pharmaceutical dosage forms.

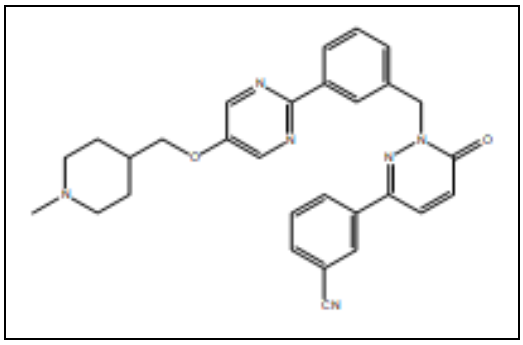

FIG. 1: STRUCTUTRE OF TEPOTINIB

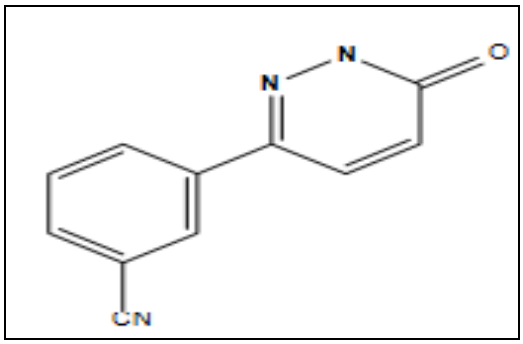

FIG. 2: STRUCTURE OF IMPURITY-1

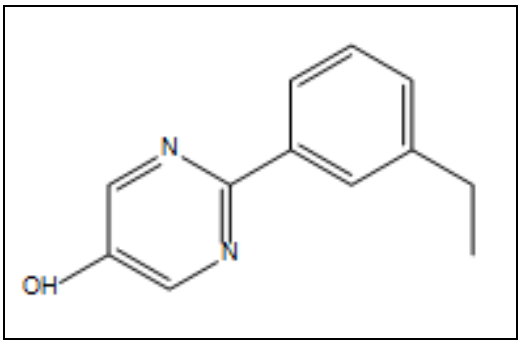

FIG. 3: STRUCTURE OF IMPURITY-2

MATERIALS AND METHOD:

Drug Samples and Chemicals: HPLC grade Acetonitrile, Ortho phosphoric acid, KH2PO4 from Rankem, MilliQ water from in-house facility was used for the entire study. The sample of Tepotinib working standard (99.85%), impurities were gifted from MERCK. Commercially available Tepmetko tablet dosage form was purchased from local pharmacy.

Instrumentation: The analysis was performed using waters Alliance HPLC system fitted with Quaternary gradient pump of e2695 series equipped with PDA detector and auto sampler injector with 10µl volume loop. Inertsil ODS C18 250x 4.6mm, 5µ column, EMPOWER 2.0 software was used for data processing, UV-Spectrophotometer-UV 1800 Schimadzu, Digital pH meter, Sonicator-Sonics and centrifugator-REMI R-8C were used.

Determination of Absorption Maxima for Tepotinib (^Max): The wavelength of maxima absorption of the drug solution and impurities (1&2) in the mixture of Acetonitrile and (0.01M) KH2PO4 (50:50)v/v pH-2.5 were scanned using PDA detector within the wavelength region of 200-400nm against Acetonitrile and (0.01M) KH2PO4 (50:50)v/v as blank the absorption curve shows isobestic point at 263nm. Thus 263nm was selected as detector wavelength for chromatographic method.

Preparation of Buffer Solution: Weigh accurately about 1.36 grams of KH2PO4 and dissolved in 1000ml milliQ water pH-2.5 was adjusted with OPA and filter through 0.22µ filter.

Preparation of Mobile Phase: Mobile phase was prepared by mixing (0.01M) KH2PO4 pH-2.5 and Acetonitrile in the ratio (50:50) v/v. It was filtered through 0.22µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of Standard Stock Solution: Weigh accurately about 40mg of Tepotinib working standard into 10ml volumetric flask. Add 7ml of diluent. Sonicate to dissolve and dilute to volume with diluent. Further pipette 1ml of the above stock

solution into a 10ml volumetric flask and dilute up to the mark with diluents (400ppm of Tepotinib)

Preparation of Sample Solution: Take 20 commercially available Tepmetko (225mg) tablets and transfer into glass motor and pestle triturate well to get fine powder from this weigh the powder containing 22.5mg equivalent weight to tepotinib and transfer into a10ml volumetric flask. Add 7ml of diluents and centrifuge for30min to dissolve and make up to the mark with diluent. Further pipette 1ml of above solution into 10 ml volumetric flask and dilute up to the mark with diluents (250ppm**)**

Preparation of Impurity Stock Solution: Weigh accurately about 10mg of impurity-1 and 15 mg impurity-2 was transferred into 10ml volumetric flask and add 7ml of diluent and sonicate up to 30min and make volume up to the mark with same diluent. Further pipette 1ml of the above solution into 10 ml volumetric flask and made up to the mark with diluent.

Spiked Impurity Mixture: Transfer 1ml of drug solution and 1ml of impurity (1&2) stock solution in 10 ml volumetric flask and add diluent up to the mark. Filter through 0.22µ membrane filter.

Method Validaton

System Suitability: System suitability test should be carried out to verify that the analytical system was working properly and can give and precise results. The working standard solution of Tepotinib and its impurity (1&2) were prepared and injected six times into HPLC system. The system suitability parameters will be evaluated from the standard chromatograms obtained by calculating the retention time, tailing factor, theoretical plates and %RSD of peak area from six replicate injections. %RSD for peak area should not be more than 2.0%, the tailing factor (T) should not be more than 2.0 and the number of theoretical plates (N) should not be less than 2000. The results obtained were as shown in the **Table 1.**

Linearity and Range: Linearity of an analytical procedure was the ability to obtain the result that is directly proportional to the amount of analyte in the sample. The linearity method was demonstrated over the concentration range of 10-60µg/ml for Tepotinib, 2.5-15µg/ml for impurity-1 and 3.75-22.5µg/ml for impurity-2.

Inject each concentration 6 times into chromatographic system and measure the average peak area (n=6). Plot a graph of average peak area versus concentration (on X-axis and on Y-axis peak area) as shown in the figure- 4 and calculate the correlation coefficient and regression equation. Correlation coefficient should not be less than 0.999. The results obtained were as shown in the **Table 2.**

Precision: The precision of analytical method was studied by analysis of multiple sampling of homogenous sample. The precision expressed in terms of relative standard deviation.

System Precision: For system precision six injections of Tepotinib working standard solution including its impurities were made each time peak area and retention time was recorded. The %RSD for the replicate injections was calculated. The results obtained were as shown in the **Table 3.**

Method Precision: For method precision six concentrations were made from same homogenous blend containing Tepotinib sample solution with its impurities, injected into the chromatographic system and the average peak area, retention time and %RSD were calculated and the results obtained were as shown in the **Table 4.**

Accuracy: The accuracy of an analytical method expresses the closeness between the true value and value found. Accuracy studies were conducted in terms of % recovery. To conduct recovery studies Tepotinib stock solution and its formulation solution were spiked with different concentrations of impurity (1&2) after analysis the % recovery and %RSD were calculated. The recovery data was shown in the **Table 5, 6.**

Specificity: Specificity of an analytical method was ability to measure specifically the analyte of interest without interference from blank, placebo and known impurities. For this purpose blank, standard, sample, chromatogram were recorded. The obtained chromatograms as shown in the **Fig. 9, 10, 11, 12.**

Robustness: As a part of robustness, deliberate change in the flow rate & mobile phase composition, the variation was made to evaluate the impact on method. The flow rate was varied at (±)

0.2% and organic content in the mobile phase was varied at (±) 2%. The working standard solution of Tepotinib & its impurities (1&2) were prepared and analysed using the varied flow rate and mobile phase composition. The results obtained were as shown in the **Table 7, 8.**

Ruggedness of Test Method: Analyst to analyst variability study was conducted by different analysts under similar conditions at different times. Two samples were prepared and each was analysed as per test method. The %RSD of peak area should not be more than 2.0%. Obtained results were as shown in the **Table 9.**

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection (LOD) limit of quantification (LOQ) of the drug was calculated by the method based on the standard deviation (Σ) and a slope of calibration curve, using the formula:

The results were as shown in the **Table 10.**

$$LOD=3.3x\sigma/S$$

$$LOQ=10x\sigma/S$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve

Forced Degradation Studies:

Acid Degradation: Pipette 1ml of drug stock solution into a 10ml volumetric flask and add 1ml of 1N HCL.

Then, the volumetric flask was kept in water bath at 60ºC for 30min, cool the flask and neutralize with 1N NaoH and make up to 10ml with diluent. Filter the solution with 0.22 micron syringe filter and inject into the chromatographic system. The obtained chromatogram was as shown in the **Fig. 15.**

Alkali Degradation: Pipette 1ml of drug stock solution into a 10ml volumetric flask and add 1ml of 1N NaoH. Then, the volumetric flask was kept in water bath at 60ºC for 30mins and neutralize with 1ml of 1N HCL and make up to 10ml with diluent. Filter the solution with 0.22 micron syringe filter and inject into the chromatographic system. The obtained chromatogram was as shown in the **Fig. 16.**

Thermal Degradation: Tepotinib sample of 10mg each was taken in two 10ml volumetric flasks and sealed. One flask was exposed to dry heat in hot air oven for specified temperature and time interval and the other was kept as control. The obtained chromatogram was as shown in the **Fig. 17.**

Peroxide Degradation: Pipette 1ml of drug stock solution into a 10ml volumetric flask,1ml of 3%v/v of hydrogen peroxide was added and the volume was made up to the mark with diluent. The volumetric flask was then kept in water bath at 60ºC for 30mins and cool the flask. Filter the solution with 0.22 micron syringe filters and inject into the chromatographic system. The obtained chromatogram was as shown in the **Fig. 18.**

Reduction Degradation: From the drug stock solution 1ml was taken in 10ml volumetric flask, add 3ml of 10% sodium bisulphate, the volumetric flask was kept in water bath at 60ºC for 30mins and the volume was made up to the mark with diluent .Filter the solution with 0.22 micron syringe filter and inject into the chromatographic system The obtained chromatogram was as shown in the **Fig. 19.**

Photolytic Degradation: Tepotinib layer of 1mm thickness was prepared in a petridish and exposed to ICH recommended photo stability conditions with the overall illumination of not less than 1.2 million lx hours along with the integrated near ultraviolet energy of not less than 200Wh/m^2. Another petridish containing the drug(1mm layer thickness) was wrapped with aluminium foil and kept as control. The obtained chromatogram was as shown in the **Fig. 20.**

Hydrolysis Degradation: From the drug stock solution 1ml was taken in 10ml volumetric flask, add 2ml of water to disperse and dissolve and heated at 60ºC for 1hour on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature and diluted to volume with diluent. Filter the solution with 0.22 micron syringe filter and inject into the chromatographic system. The obtained chromatogram was shown in the **Fig. 21.**

RESULTS AND DISCUSSION: Several columns and mobile phases were tested for the estimation of Tepotinib in presence of its known impurities using

mobile phases like acetonitrile and 0.1%TEA (80:20, 70:30, 75:25, 60:40)v/v, acetonitrile and 0.1% formic acid (90:10, 80:20)v/v, acetonitrile and (0.01M) KH2PO4 (30:70, 40:60, 45:55) and stationary phases like Waters symmetry C18(150x4.6mm,3.5µ), X-bridge phenyl (250x4.6mm, 5µ). Hence Inertsil ODS C18 (250x4.6mm, 5µ) column was suitable for the proposed method.

Method Development: In setting up the condition for development of the assay method, the choice of the detection wavelength was based on the scanned absorption spectrum for Tepotinib and its known impurities.

The prepared solution was loaded into the auto sampler and system was set in order to take the auto injection in HPLC with PDA detector. The obtained spectra was as shown in the **Fig. 4**. From the below spectra of both drug and impurities, a wavelength was selected at which the drug and impurities showed maximum absorbance at 263nm.

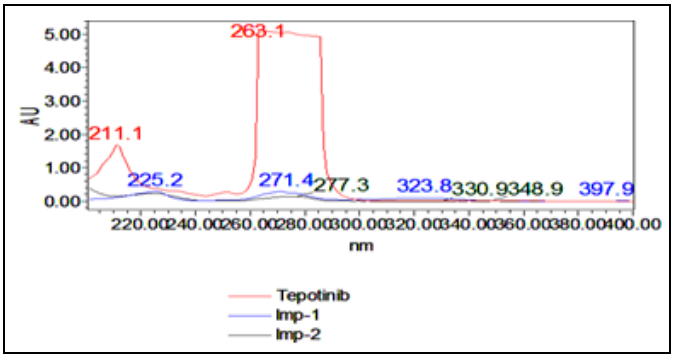

FIG. 4: PDA SPECTRUM

Selection of Chromatographic Method: The choice of chromatographic method is based on the nature of sample, its molecular weight and solubility. As drug is polar in nature, the reverse phase chromatographic technique was selected for present work. The obtained optimized chromatogram was shown in **Fig. 5.**

Optimized Chromatographic Conditions Column: Inertsil ODS C18 (250x4.6mm.5µ).

Mobile Phase: Acetonitrile and (0.01M) KH2PO4-(50:50)v/v Injection volume: 10µl

Flow Rate: 1.0ml/min Detection wavelength: 263nm Run time: 6 min.

Retention time for Tepotinib: 2.565min Retention time for impurity-1: 3.471min Retention time for impurity-2: 4.508 min.

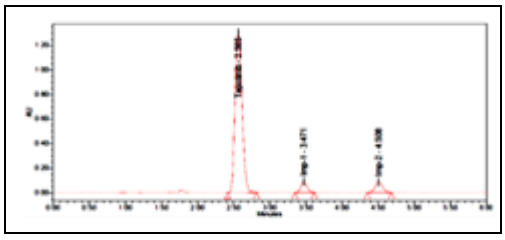

FIG. 5: OPTIMIZED CHROMATOGRAM

Method Validation: The validation of RP-HPLC method for the determination of Tepotinib, impurity-1 and impurity-2 as per the protocol and to demonstrate that the method was appropriate for its intended use and studied for the following parameters. All the validation parameters were carried out according to ICH guidelines.

System Suitability: The system suitability was done by injecting working standard solution of Tepotinib and its impurities six times into HPLC system, as shown in **Table 1**. Hence, it was concluded that the instrument, reagents and column were to perform validation.

TABLE 1: RESULTS OF SYSTEM SUITABILITY

Parameter n=6	Tepotinib	Impurity-1	Impurity-2
Retention time (min)	2.565	3.471	4.508
Theoretical plates	3247	7997	8507
Tailing factor	1.17	1.15	1.05
USP Resolution	-	4.51	6.23
%RSD of peak areas	0.23	0.44	0.29

Linearity and Range: The aliquots of the standard spiked solution were prepared and injected into HPLC system. The correlation coefficient was within the limits for Tepotinib, impurity-1 and impurity-2, the obtained regression equations were y = 22186x + 88871, y = 52077x + 124.36, y = 43433.67x + 630.25 respectively was as shown in the **Fig. 6, 7, 8** and **Table 2.**

TABLE 2: LINEARITY RESULTS OF TEPOTINIB, IMPURITY-1, IMPURITY-2

S. no.	Concentration(µg/ml)			Average peak area(n=6)		
	Tepotinib	Impurity-1	Impurity-2	Tepotinib	Impurity-1	mpUrity-2
1	10	2.50	3.75	2305479	150320	175274
2	20	5.00	7.50	4459861	272621	325402
3	30	7.50	11.25	7140237	390218	476528
4	40	10.00	15.00	8812364	542145	646547
5	50	12.50	18.75	11105415	672651	813248
6	60	15.00	22.50	13389585	777156	987814

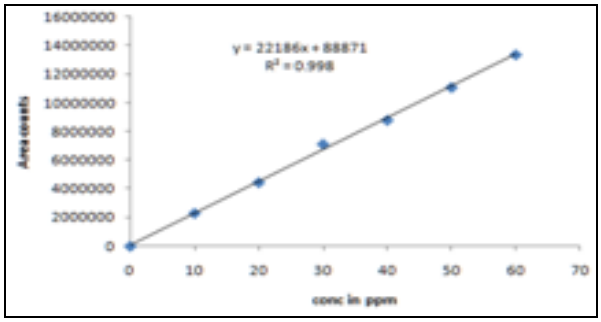

FIG. 6: CALIBRATION CURVE OF TEPOTINIB

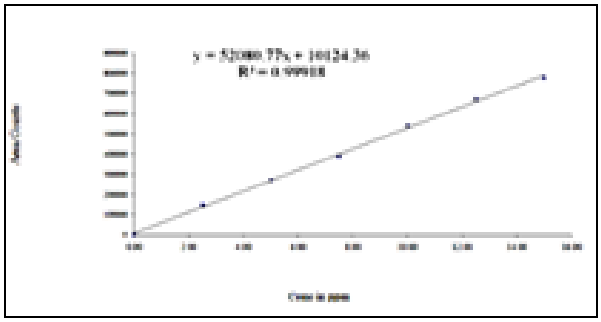

FIG. 7: CALIBRATION CURVE OF IMPURITY-1

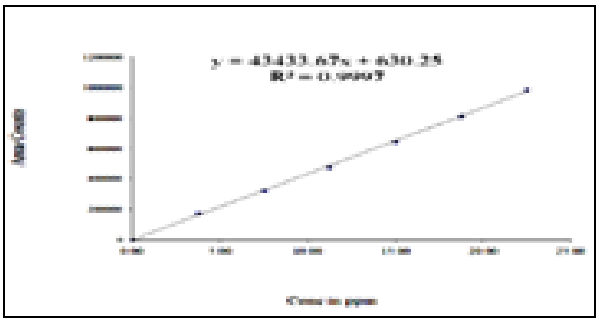

FIG. 8: CALIBRATION CURVE OF IMPURITY-2

Precision Studies: System precision and method precision studies were conducted. The %RSD calculated for system and method precision was<2.0%. The results of system suitability and method precision revealed that the method was precise as shown in the **Table 3, 4.**

TABLE 3: SYSTEM PRECISION VALUES FOR TEPOTINIB, IMPURITY-1, IMPURITY-2

S. no.	Peak area		
	Tepotinib	Impurity-1	Impurity-2
1	8822928	531542	641033
2	8836784	536048	645324
3	8829758	532167	646102
4	8840136	534490	642584
5	8802785	537841	644652
6	8862984	535064	643258
Mean (n=6)	8832562	534525	643825
%RSD	0.23	0.44	0.29

TABLE 4: METHOD PRECISION VALUES FOR TEPOTINIB, IMPURITY-1 AND IMPURITY-2

S. no.	Peak area		
	Tepotinib	Impurity-1	Impurity-2
1	8862389	532634	642365
2	8844642	535218	640786
3	8808640	534156	643625
4	8823039	529689	648572
5	8870326	530231	647304
6	8897951	537032	646258
Mean(n=6)	8851164	533160	644181
%RSD	0.37	0.54	0.47

Accuracy: The recovery data showed that the proposed method was accurate. The results were found to be within the limits, as given in the **Table 5, 6.**

TABLE 5: RECOVERY DATA FOR TEPOTINIB

	Spiked levels								
		50%			100%			150%	
Imurity-1	100.4	100.1	99.9	99.8	100	99.8	99.3	99	99.7
Mean recovery		100.1%			99.8%			99.3%	
%RSD		0.70			0.65			0.35	
Impurity-2	100.6	100.8	100.6	100	99	101	100	99.9	100
Mean % recovery		100.6			100%			99.9%	
%RSD		0.64			0.40			0.20	

TABLE 6: RECOVERY DATA FOR TEPOTINIB TABLET DOSAGE FORM

	Spiked levels								
		50%			100%			150%	
Imurity-1	99.9	100.4	100.1	100	99.8	99.8	99.7	99	99.3
Mean %recovery		100.1%			99.8%			99.3%	
%RSD		0.70			0.65			0.35	
Impurity-2	100.8	100.6	100.6	101	100	99	100	99.9	100
Mean %recovery		100.6			100%			99.9%	
%RSD		0.64			0.40			0.20	

Specificity: Solution of standard and sample should be identical with near retention times, as shown in the **Fig. 9, 10, 11, 12**. The chromatogram of standard and sample were identical. There was no interference with blank and placebo at retention time of analyte hence the method was specific.

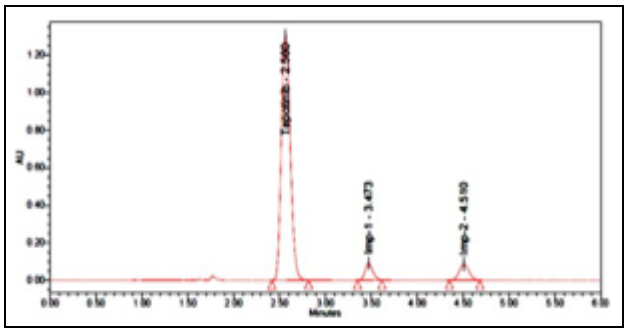

FIG. 9: CHROMATOGRAM OF TEPOTINIB, IMPURITY-1, IMPURITY-2 (STANDARD)

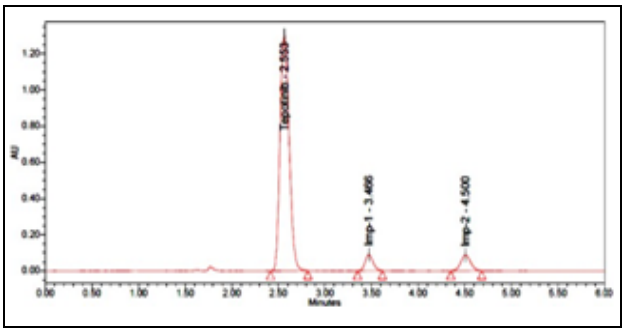

FIG. 10: CHROMATOGRAM OF TEPOTINIB, IMPURITY-1, IMPURITY-2(SAMPLE)

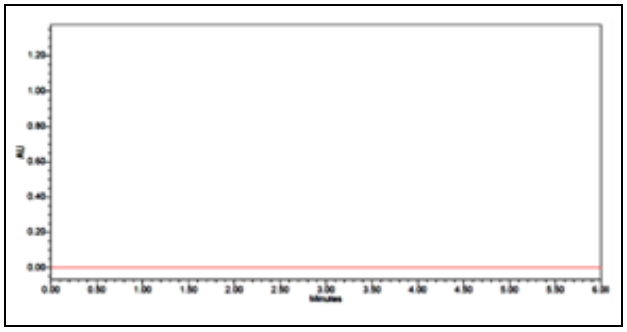

FIG. 11: CHROMATOGRAM OF BLANK

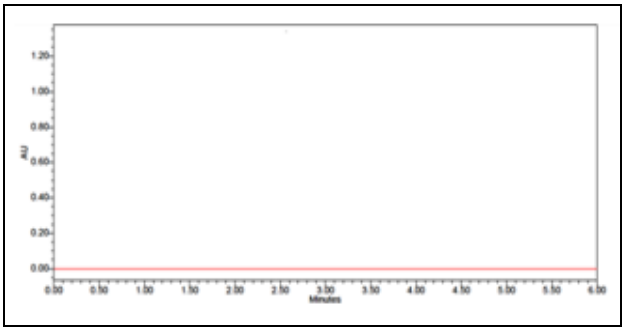

FIG. 12: CHROMATOGRAM OF PLACEBO

Robustness: The analysis of sample was conducted in the slight altered conditions of optimized conditions and observed for the peak area and retention time. The altered conditions include variation in flow rate (0.2ml/min) and variation in organic content of the mobile phase composition (2.0%v/v) as given in the **Table 7, 8**. The %RSD was within limits hence the method was robust.

Effect of Variation of Flow Rate:

TABLE 7: ROBUSTNESS DATA OF TEPOTINIB, IMPURITY-1, IMPURITY-2

	Tepotinib			Impurity-1			Impurity-2		
S. no.	Flowrate	Retentiontime	Peakarea	Flowrate	Retention time	Peakarea	Flow rate	Etention time	Peak area
1	0.8	2.850	9063777	0.8	3.859	578868	0.8	5.402	728941
2	0.8	2.854	9051264	0.8	3.855	573645	0.8	5.408	722369
3	0.8	2.855	9026556	0.8	3.857	577855	0.8	5.406	725637
%RSD			0.21			0.48			0.45
1	1.2	2.167	7332097	1.2	2.918	421207	1.2	3.800	533341
2	1.2	2.167	7306792	1.2	2.920	424903	1.2	3.805	532976
3	1.2	2.167	7359762	1.2	2.914	422578	1.2	3.803	536564
%RSD			0.36			0.44			0.37

Effect of Organic Phase composition:

TABLE 8: ROBUSTNESS DATA OF TEPOTINIB, IMPURITY-1, IMPURITY-2

	Tepotinib			Impurity-1			Impurity-2		
S. no.	Composition	Retention time	Peakarea	Composition	Retention time	Peak area	Composition	Retention time	Peak area
1	48:52	2.352	8592306	48:52	3.157	468508	48:52	3.847	628459
2	48:52	2.356	8579585	48:52	3.156	466254	48:52	3.845	625638
3	48:52	2.351	8523590	48:52	3.152	462603	48:52	3.842	623064
%RSD			0.43			0.64			0.43
1	52:48	3.108	1.688389	52:48	4.225	645353	52:48	4.472	738000
2	52:48	3.104	10703547	52:48	4.222	642968	52:48	4.476	732458
3	52:48	3.106	10423697	52:48	4.220	640235	52:48	4.479	735789
%R SD			1.48			0.40			0.38

Ruggedness (Analyst to Analyst Variability): Two samples were prepared and each was analysed by different analysts as per the test method. The %RSD was found to be within the limits as given in **Table 9.**

TABLE 9: RUGGEDNESS DATA (EFFECT OF CHANGE IN ANALYST)

Analyst	Peak area of Tepotinib	Peak area of impurity-1	Peak area of impurity-2
Analyst-1	8824632	535632	640362
Analyst-2	8856387	539368	645267
Mean	8840509	537500	642814
SD	22454.18	2641.75	3468.36
%RSD	0.25	0.49	0.54

LOD and LOQ: The LOD and LOQ were calculated as per formula and were shown in the below **Table 10** and the results obtained were within the limits.

TABLE 10: LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Sample	LOD (s/n)	LOQ
Tepotinib	0.8µg/ml	2.7µg/ml
Imp-1	0.4µg/ml	1.2µg/ml
Imp-2	0.5µg/ml	1.8µg/ml

Assay: The result of marketed formulation showed good agreement with labelled claim using the processed method. The results obtained were as shown in the **Table 11.** The obtained chromatogram was as shown in the **Fig. 13.**

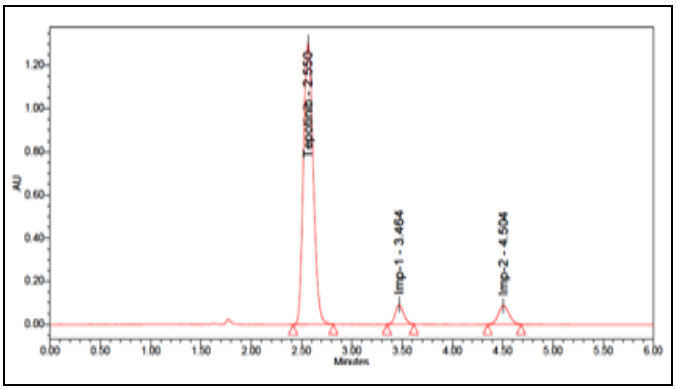

FIG. 13: CHROMATOGRAM OF ASSAY

TABLE 11: ASSAY RESULTS FOR TEPOTINIB

Brand	Drug	Sample area	Average area (n=6)	Labelled weigh t inmg	Estimated amount	%of drug content	Std purity	Amount found (µg/ml)	%Assay
Tepmet ko	Tepoti nib	8814456 8853951	8834204	25	24.75	99	99.9	40.25	100.3

Forced Degradation Studies: The results obtained from forced degradation studies revealed that Tepotinib was degraded more in alkaline condition than acid, thermal, peroxide, reduction, photolytic, hydrolysis. Purity angle and purity threshold were also calculated. The results obtained were as shown in the **Table 12** the chromatograms were shown below [14, 15, 16, 17, 18, 19, 20, 21].

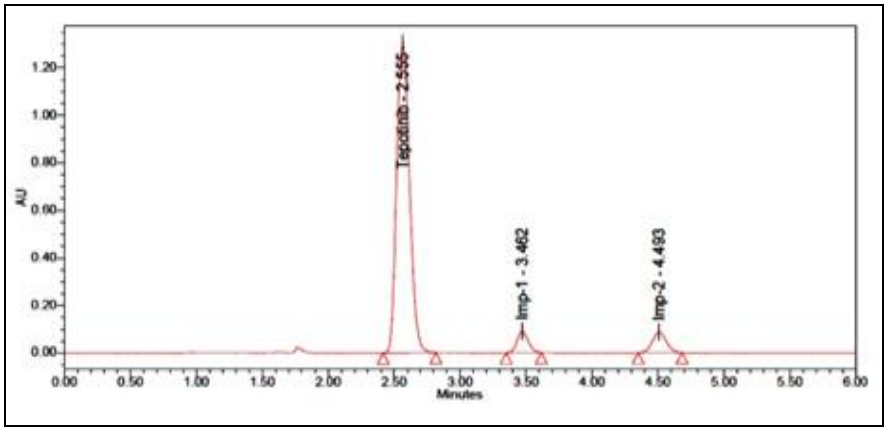

FIG. 14: REPRESENTATIVE CHROMATOGRAM FOR CONTROL

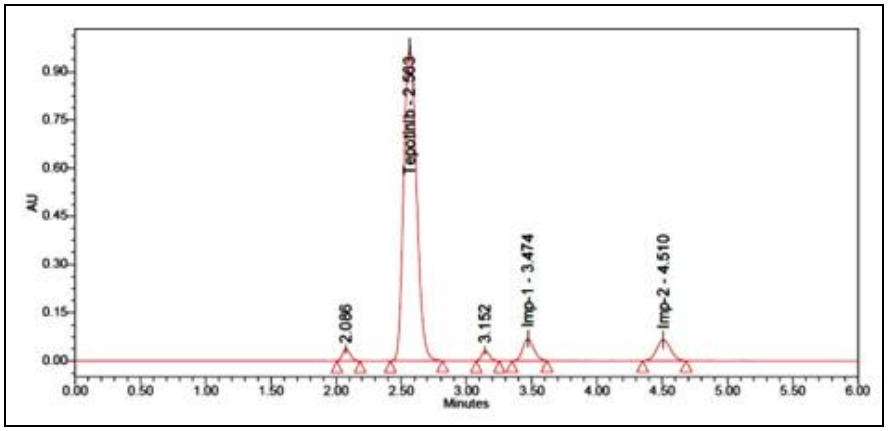

FIG. 15: REPRESENTATIVE CHROMATOGRAM FOR ACID DEGRADATION

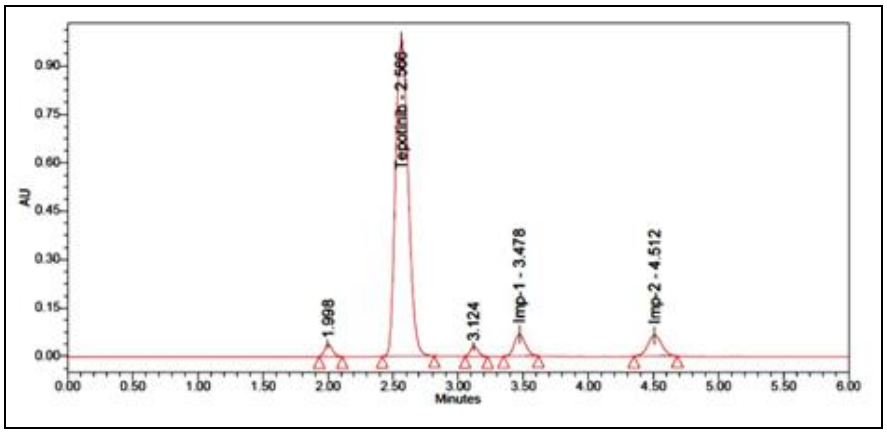

FIG. 16: REPRESENTATIVE CHROMATOGRAM FOR ALKALI DEGRADATION

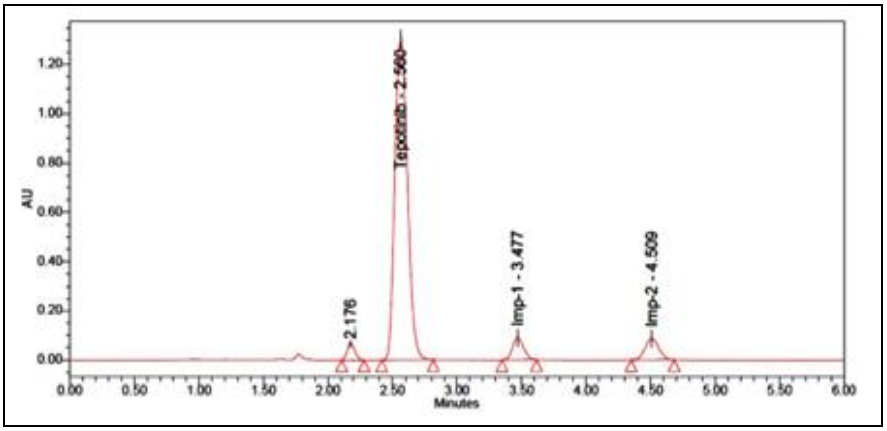

FIG. 17: REPRESENTATIVE CHROMATOGRAM FOR THERMAL DEGRADATION

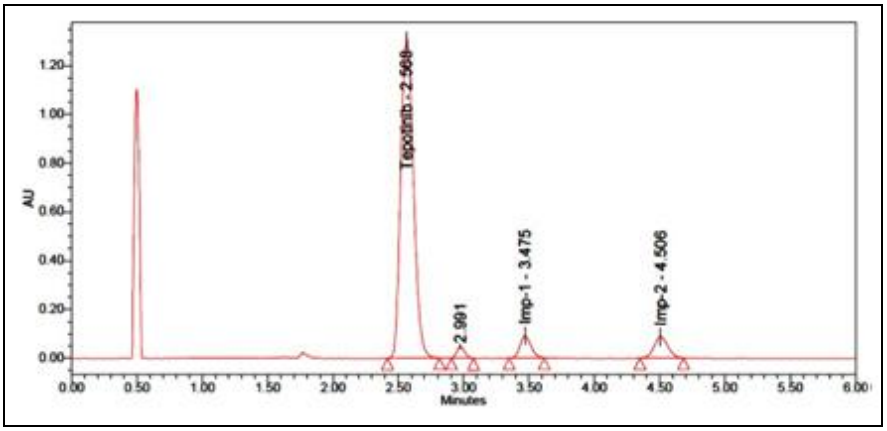

FIG. 18: REPRESENTATIVE CHROMATOGRAM FOR PEROXIDE DEGRADATION

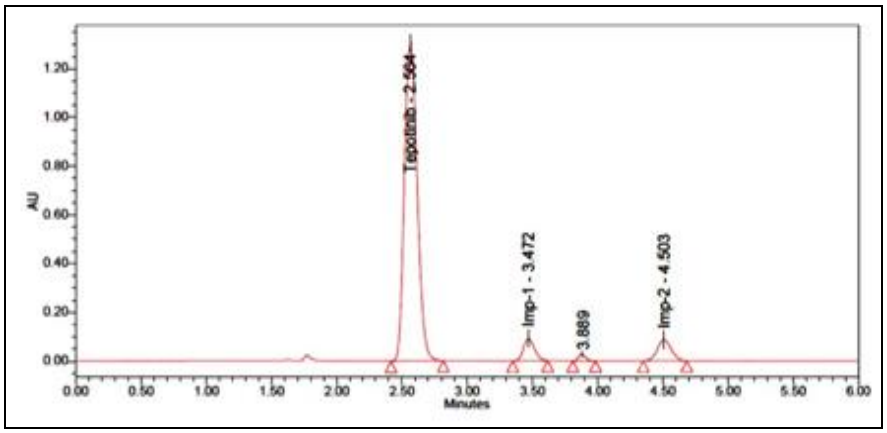

FIG. 19: REPRESENTATIVE CHROMATOGRAM FOR REDUCTION DEGRADATION

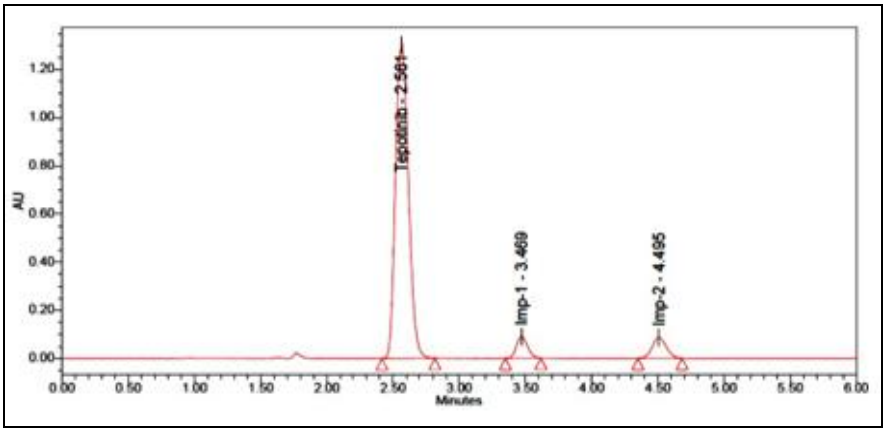

FIG. 20: REPRESENTATIVE CHROMATOGRAM FOR PHOTOLYTIC DEGRADATION

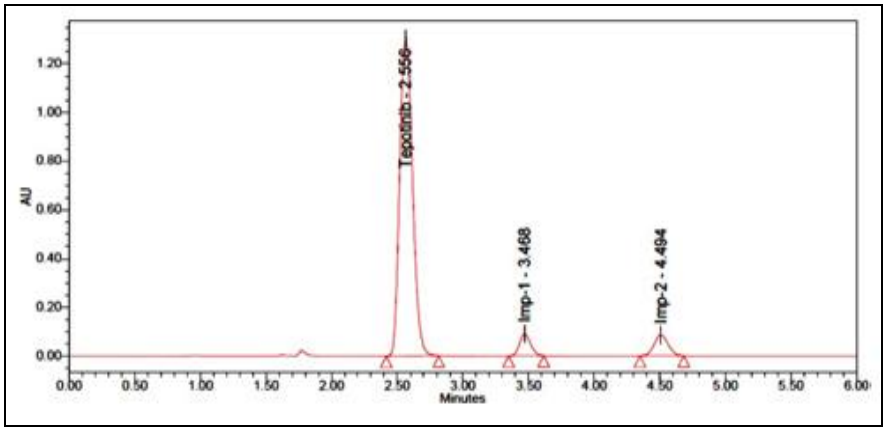

FIG. 21: REPRESENTATIVE CHROMATOGRAM FOR HYDROLYSIS DEGRADATION

TABLE 12: RESULTS FOR FORCED DEGRADATION STUDY FOR TEPOTINIB

Stress conditions	% of assay after degradation	Purity Angle	Purity threshold	%Degradation
Control	100	0.134	1.228	0
Acid	85.4	0.137	1.222	14.6
Alkali	84.9	0.133	1.229	15.1
Thermal	86.9	0.147	1.265	13.1
Peroxide	87.4	0.129	1.235	12.6
Reduction	89.7	0.138	1.223	10.3
Photolytic	99.7	0.132	1.284	0.3
Hydrolysis	99.9	0.128	1.273	0.1

CONCLUSION: The developed RP-HPLC method for the estimation of selected drug Tepotinib in presence of its impurities was simple, rapid, accurate, precise, robust and economical.

The mobile phase and solvents were simple to prepare and economical. Forced degradation studies revealed that the proposed method was stability indicating and used for stability studies.

All the validation parameters and results were found within the acceptable limits as given in the validation protocol.

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