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**Research Article**

# NEW STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF TEPOTINIB IN BULK AND TABLET DOSAGE FORM BY USING QBD APPROACH

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## ABSTRACT:

The Implementation of Quality by Design (QbD) principles in High-Performance Liquid Chromatography (HPLC) analysis ensures a systematic and scientific approach to method development, optimization, and validation. This approach focuses on understanding the relationship between Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs), enabling the design of robust and reliable analytical methods. By utilizing Design of Experiments (DoE), risk assessment, and robustness testing, QbD in HPLC facilitates the identification of optimal method conditions, ensuring accurate and precise results. This methodology enhances method transferability, scalability, and regulatory compliance, ultimately leading to improved product quality and reduced development timelines. The application of QbD in HPLC analysis demonstrates a proactive and holistic approach to analytical method development, aligning with regulatory guidelines and industry best practices So a simple, accurate, precise method was developed for the estimation of the Tepotinib in bulk and pharmaceutical dosage form by using QbD approach was developed. Chromatogram was run through Ascentis C18 Column, 100Å, 10 µm, 4.6 mm X 150 mm. Mobile phase containing 0.01N Kh2PO4: Acetonitrile taken in the ratio 55.3: 44.7(%v/v) was pumped through column at a flow rate of 0.95 ml/min. Temperature was maintained at 27.8°C. Optimized wavelength selected was 235nm. Retention time of Tepotinib was found to be 2.422 min. %RSD of the Tepotinib were and found to be 0.4 and 0.3 respectively. %Recovery was Obtained as 99.94% for Tepotinib. LOD, LOQ values were obtained from regression equations of Tepotinib were 0.02ppm, 0.06 ppm respectively. Regression equation of Tepotinib is y = 41599x + 9224.1.

**Key Words:** Tepotinib, RP-HPLC, QbD Approach, RP-HPLC, Central Composite Design

**Introduction:**

**CENTRAL COMPOSITE DESIGN**

A $3^2$ full factorial design was utilized to optimize the RP-HPLC method for the simultaneous detection of Tepotinib, emphasizing on two independent variables: the flow rate and the methanol content in the mobile phase. The flow rate was evaluated at 0.9, 0.95, and 1.0 mL/min, and the methanol concentration was altered to three different levels: 35%, 45%, and 45% (v/v with Phosphate buffer). This setup resulted in twenty experimental runs, which allowed for a thorough evaluation of how variations in methanol concentration and flow rate affected significant chromatographic responses, including tailing factor, theoretical plate count, and retention duration. The factorial design technique assured a procedure that is resilient and trustworthy in conformity with QbD principles by making it easier to find the appropriate chromatographic parameters and providing insights into the interaction between the two variables. The experimental data was analyzed using surface plots and ANOVA, which made it easier to see how flow rate and methanol concentration interacted while determining the statistical significance of each element in connection to the chromatographic responses. Tepotinib is a highly selective, orally bioavailable small-molecule inhibitor of the hepatocyte growth factor receptor (c-MET), developed to target malignancies driven by dysregulation of the MET proto-oncogene [1]. Aberrant activation of the MET pathway—via genetic amplification,

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overexpression, point mutations, or most notably MET exon 14 (METex14) skipping alterations—plays a crucial role in tumor growth, epithelial-mesenchymal transition, invasion, and metastasis [2]. METex14 skipping produces loss of the juxtamembrane domain responsible for MET receptor degradation, resulting in sustained oncogenic signaling and uncontrolled cellular proliferation [3]. These findings led to a strong therapeutic rationale for precision inhibition of MET in selected cancer populations. Tepotinib acts as an ATP-competitive, reversible MET tyrosine kinase inhibitor, blocking downstream signaling cascades including RAS/MAPK, PI3K/AKT, STAT3, and NF-κB, which are vital regulators of survival, angiogenesis, and motility in MET-driven cancers [4]. Preclinical studies demonstrated that tepotinib selectively inhibits MET phosphorylation and MET-dependent tumor growth while sparing non-MET-driven pathways, supporting its advancement into clinical development [5]. Clinical activity was validated through the global VISION trial, a pivotal multicohort, open-label Phase II study evaluating tepotinib in patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC) harboring METex14 skipping alterations [6]. The trial demonstrated durable objective responses, clinically meaningful progression-free survival, and quality-of-life preservation, including in both treatment-naïve and previously treated patients [6]. Importantly, VISION incorporated tissue-based and liquid biopsy approaches for molecular identification, demonstrating similar response outcomes and establishing plasma-based diagnostics as a valid alternative when tumor biopsy is not feasible [7]. These results strongly contributed to regulatory approvals worldwide. Tepotinib achieved its first global approval in Japan in March 2020 as the first targeted therapy specifically for NSCLC with METex14 skipping [8]. In the United States, the Food and Drug Administration (FDA) granted accelerated approval in February 2021, later converting it to full approval after confirmatory evidence from extended VISION analyses [9]. The drug is additionally authorized across Europe and other regions with labeling that reinforces biomarker-based patient selection [10]. The recommended dose of tepotinib for metastatic NSCLC is 450 mg orally once daily with food, administered as two 225-mg tablets, until unacceptable toxicity or disease progression [9]. Dose reductions and interruptions are guided by toxicity management protocols detailed in the product label [9]. Tepotinib's safety profile is manageable and consistent across regulatory datasets. The most frequently observed adverse event is peripheral edema, attributed to MET pathway involvement in endothelial barrier function [11]. Other common adverse reactions include nausea, fatigue, diarrhea, musculoskeletal pain, decreased appetite, and dyspnea [11]. Although infrequent, serious toxicities such as drug-induced interstitial lung disease/pneumonitis and hepatotoxicity require monitoring and prompt intervention [12]. Overall, discontinuation rates remain low and dose adjustments effectively maintain treatment continuity in most patients [11,12]. Comparative research between tepotinib and other MET inhibitors—particularly capmatinib—is accelerating. While both demonstrate potent activity against METex14-altered NSCLC, real-world studies suggest differences in edema burden and cross-trial variability in response kinetics, although head-to-head trials are not yet available [13]. Moreover, increasing evidence shows activity in central nervous system (CNS) metastases, an important determinant in long-term disease control [14]. Outside NSCLC, ongoing investigations are probing tepotinib's potential in MET-dysregulated tumors such as hepatocellular carcinoma, gastric cancer, and colorectal cancer, including in combination with immunotherapy and anti-angiogenic regimens [15].

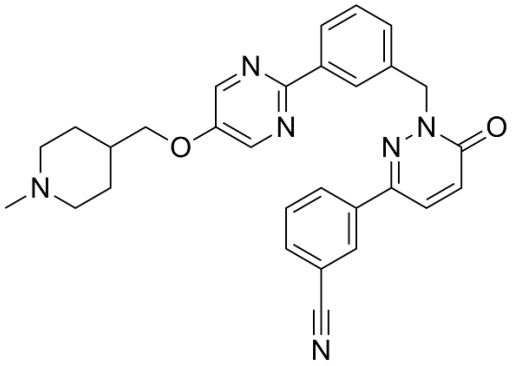



**Figure 1: Structure of Tepotinib**

A QbD is defined as "A systemic approach to the method development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management5." The QbD approach emphasizes product and process understanding with quality risk management and controls, resulting in higher assurance of product quality, regulatory flexibility, and continual improvement. The QbD method was based on the understanding and implementation of guidelines ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality System[16,17,18]. Analytical science is considered to be an integral part of pharmaceutical product development and hence go simultaneously during the entire product life cycle. Analytical QbD defined as a science and risk-based paradigm for analytical method development, endeavouring for understanding the predefined

objectives to control the critical method variables affecting the critical method attributes to achieve enhanced method performance, high robustness, ruggedness, and flexibility for continual improvement[19,20]. Extensive literature research has unearthed a multitude of recorded analytical procedures[21-31], including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for HPLC approach. Hence, a reliable and cost-effective approach is suggested for assessing the QbD and stability of Tepotinib, and their medicinal dose form using RP-UPLC must be validated and developed as per ICH guidelines.

**Materials and Methods:**

Tepotinib (API), and Tepotinib tablet (Tepmetko), Acetonitrile, Methanol, Ortho Phosphoric Acid, Distilled water. All of the solvents and chemicals were of HPLC quality and obtained from Rankem Chemicals Pvt Ltd.

Instrumentation:

The Method Development and Validation was performed by Waters HPLC Model 2695 equipped with PDA Detector and Empower 3 Software. For QbD Design Expert 13 Software, Analytical weighing Balance, Ultrasonicator, pH Meter, Hot air oven.

API Formulation

Preparation of Standard stock solutions and Working Solution: Accurately weighed 11.25mg of Tepotinib, and transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (225µg/ml of Tepotinib) from this sol 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (22.2 µg/ml of Tepotinib).

Sample Formulation

Preparation of Sample stock solutions and Working Solution: 10 tablets were weighed, powdered and then Weight equivalent to one tablet (545.87 Avg wt) was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (2250µg/ml of Tepotinib) From this 0.5ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (22.5µg/ml of Tepotinib).

**Method Validation**

The proposed approach is validated using ICH criteria for the aim of validating analytical procedures. Validation metrics included system suitability, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, robustness, specificity, and degradation studies.

**System suitability parameters:**

Following six injections of the working standard solution into the HPLC system, the chromatographic analysis was performed using the planned and optimised parameters. The system suitability parameters were determined by calculating the percentage RSD of retention times, theoretical plates, and peak areas from six duplicate injections.

**Specificity:** Interference in the optimised approach is checked. Using this strategy, we shouldn't observe interfering peaks in blank and placebo during these medications' retention times. Thus, this approach was described as being particular.

**Precision:**

The precision of the devised analytical method was evaluated using repeatability (intraday) and intermediate precision (inter-day). Repeatability is the use of an analytical procedure in a lab over a short period of time that was assessed by assaying the samples on the same day. By comparing the assays across a number of days, intermediate precision was evaluated. SD and %RSD were calculated.

**Linearity:**

Stock solution I was repeatedly diluted volume to volume over the range of 5.6-33.8 µg/ml for Tepotinib in order to construct standard calibration curves with six different concentrations, including the LOD and LOQ. Peak area and drug concentration were calibrated using linear curves. Using linear regression, which was computed using the least squares regression approach, the linearity was examined.25 µg/mL: Take 0.25 mL of stock solution and dilute to 10 mL

- 50 µg/mL: Take 0.5 mL of stock solution and dilute to 10 mL
- 75 µg/mL: Take 0.75 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 1.0 mL of stock solution and dilute to 10 mL
- 125 µg/mL: Take 1.25 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 1.5 mL of stock solution and dilute to 10 mL

**Accuracy:**

Accuracy was performed in triplicate for various concentrations of Tepotinib equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. Dilution were as follows.

- 50 µg/mL: Take 0.1 mL of stock solution and dilute to 10 mL
- 100 µg/mL: Take 0.2 mL of stock solution and dilute to 10 mL
- 150 µg/mL: Take 0.3 mL of stock solution and dilute to 10 mL

**Sensitivity:**

**Limit of detection and Limit of Quantification**

Limits of detection (LOD) and limit of quantitation (LOQ) were established using the signal-to-noise ratio. The detection limit was stated to as the lowest concentration level resulting in a peak area of three times the baseline noise. The lowest concentration level that produced a peak area with a signal-to-noise ratio greater than ten was referred to as the quantitation limit.

Based on the response's standard deviation and calibration curve's slope, the LOD and LOQ can be estimated. The formulae given below can be used to calculate LOD and LOQ:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

where S is calibration curve of the slope and σ is the response of the standard deviation.

**Sensitivity Stock solution:** Take 0.25 mL of stock solution and dilute to 10 mL

- **LOD:** From above take 0.3 ml solution and dilute to 10 mL
- **LOQ:** From above take 0.9 ml solution and dilute to 10 mL

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

**Assay:**

For the brand (Tepmetko) with label claims of Tepotinib 225 mg, the assay and percentage purity were computed. The value that was determined was compared against that of standard value without interference from the excipients used in the tablet dosage form

**Degradation Studies**

These studies describe the stability of the pure pharmaceutical substance under different stress conditions and are helpful in determining the optimal storage conditions. Base, peroxide, acid, neutral hydrolysis, light, and heat degradation are all included in these studies.

**Oxidation:**

After adding 1ml of a stock solution to 10ml of a 20% volume fraction of H2O2 and allowing it to sit in an oven at 60°C for 30 minutes, a chromatogram was produced by injecting a 22.5 µg/ml solution at 10 µl into HPLC

**Acid Degradation Studies:**

1 ml of hydrochloric acid was added to 10 ml of vf with 1 ml stock and refluxed for 30 minutes at 60 $^{o}$C. A 22.5 µg/ml solution was injected at 10 µl into the system, resulting in the formation of a chromatogram.

**Alkali Degradation Studies:**

A mixture of 1 ml of stock and 1 ml of NaOH in 10 ml of vf was refluxed for 30 minutes at 60°C. A 22.5 µg/ml solution was injected at 10 µl into HPLC, resulting in the production of a chromatogram.

**Dry Heat Degradation Studies:**

The stock solution was allowed to undergo thermal deterioration in an oven set at 105oC for 6 hours. Subsequently, a chromatogram was prepared by injecting a 22.5 µg/ml solution at 10 µl into HPLC.

**Photo Stability studies:**

The stock underwent degradation by exposure to UV radiation in the laboratory for a duration of 7 days. Upon injecting a 22.5 µg/ml solution at a volume of 10 µl into HPLC, a chromatogram was developed.

**Neutral Degradation Studies:**

After refluxing the stock for 6 hours at 60 degrees Celsius, a chromatogram was prepared by injecting a 22.5 µg/ml solution at 10 µl into HPLC.

## Result and Discussion

**Parameter Selection**

Various preliminary HPLC trials were carried out for selection of Column and organic modifier. The choice of C18 column based on the preliminary investigation was done using **Ascentis C18 (150×4.6 mm, 5.0µm)**, Selection of a suitable organic modifier is also important to get better selectivity with adequate separation of all analytes.

Commonly used organic solvents for the reversed phase HPLC include Acetonitrile and Methanol, from that trials Acetonitrile showed to be an ideal and suitable organic modifier compared to Methanol, because Tepotinib was solubilized in acetonitrile compare to methanol. Therefore, **Acetonitrile was selected and finalized as the organic modifier for further optimization study**.

**Optimization of method**

The method was optimized via Central composite design. The earliest trials are needed to optimize the final approach. It was necessary to tune the organic concentration, flow rate, and column temperature. In order to maximize these characteristics, which were adjusted over three levels (high, mid, and low), central composite design was employed. different ranges of parameters ranging from **35-45% Aqueous Phase**, **temperature 27 $^0$C - 33 $^0$C** and **0.9-1.10ml/min flow rate** respectively were taken and counter and 3D surface plot showing the effect of each parameter on Retention Time, Theoretical plates and Resolution (CQA) were generated. A desirability function used to the optimal settings to estimate retention period, asymmetry, theoretical plates

**Table 1 Optimized Condition**

| ***Parameter*** | ***Condition*** |
|---|---|
| ***Mobile phase*** | *Acetonitrile: Phosphate Buffer(44:56 v/v)* |
| ***Flow rate*** | *0.9 ml/min* |
| ***Column*** | *Ascentis C18 (4.6 x 250mm, 5µm)* |
| ***Detector wave length*** | *235nm* |
| ***Column temperature*** | *30°C* |
| ***Injection volume*** | *10µL* |
| ***Diluent*** | *Water and Acetonitrile in the ratio 50:50* |

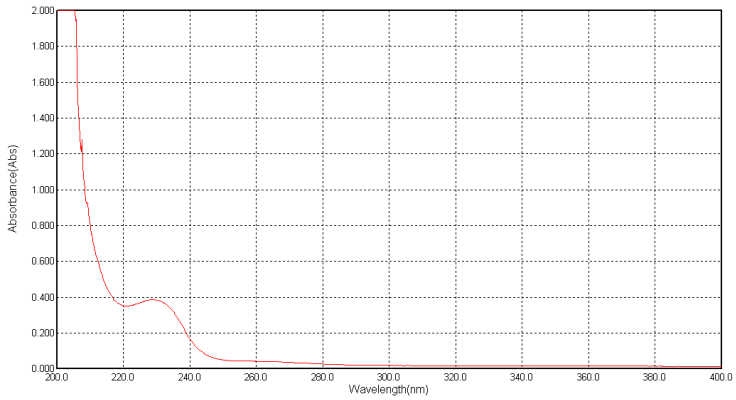



**Figure 2: UV Spectrum**

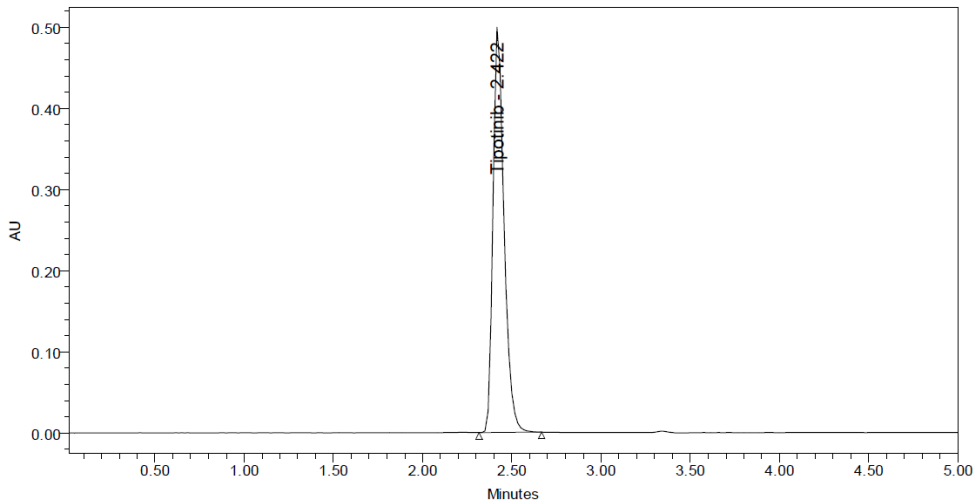



**Figure 3: Optimized Chromatogram**

## Table 2 Design summary of CCD

| *Design Summary* | | | | | |
|---|---|---|---|---|---|
| ***File version: DX 13.0.0<br>Study Type: Response surface<br>Design Type: central composite design<br>Design Model: Quadratic*** | | | ***ATP: Robustness<br>CQA: Retention time, Theoretical plates and Tailing factor<br>Runs: 20*** | | |
| ***CMPs*** | ***Unit*** | *Type* | *Subtype* | ***Min.*** | ***Max.*** |
| ***Aqueous Phase*** | ***%*** | *Numeric* | *Continuous* | ***31.59 %*** | ***48.41 %*** |
| ***Flow rate*** | ***ml/min*** | *Numeric* | *Continuous* | ***0.83 ml/min*** | ***1.17 ml/min*** |
| ***Temp*** | ***$^0$C*** | *Numeric* | *Continuous* | ***24.95 $^0$C*** | ***35.05 $^0$C*** |

## Table 3 Factors

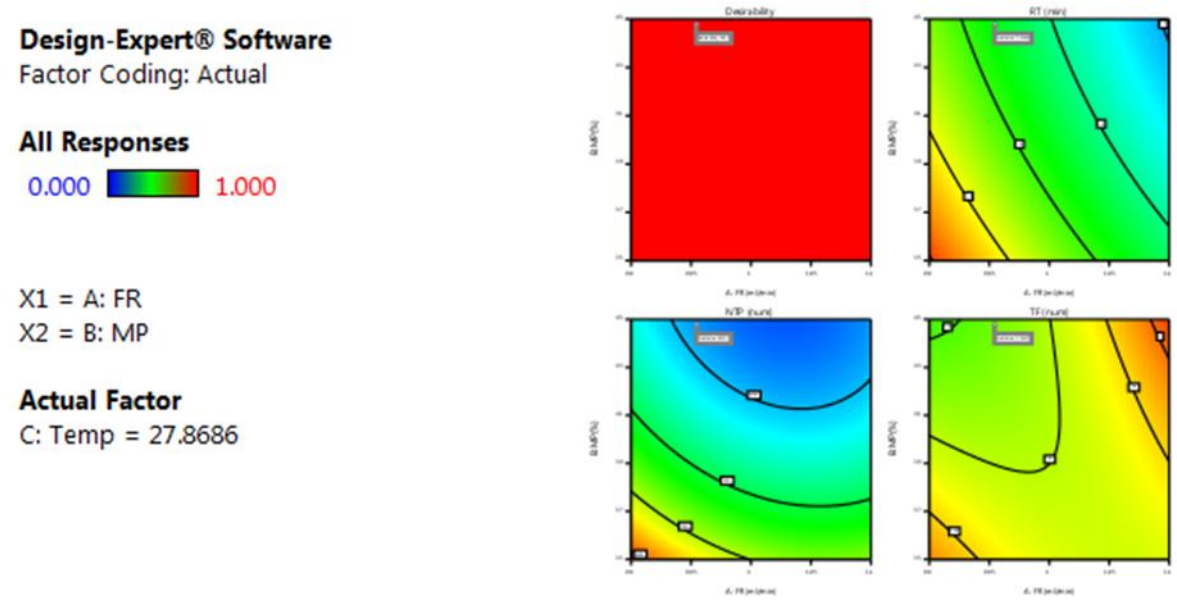
| *Factor* | *Name* | *Level* | *Low Level* | *High Level* | *Std. Dev.* | *Coding* |
|---|---|---|---|---|---|---|
| *A* | *FR* | *0.95* | *0.90* | *1.10* | *0.0000* | *Actual* |
| *B* | *MP* | *44.78* | *35.00* | *45.00* | *0.0000* | *Actual* |
| *C* | *Temp* | *37.87* | *27.00* | *33.00* | *0.0000* | *Actual* |

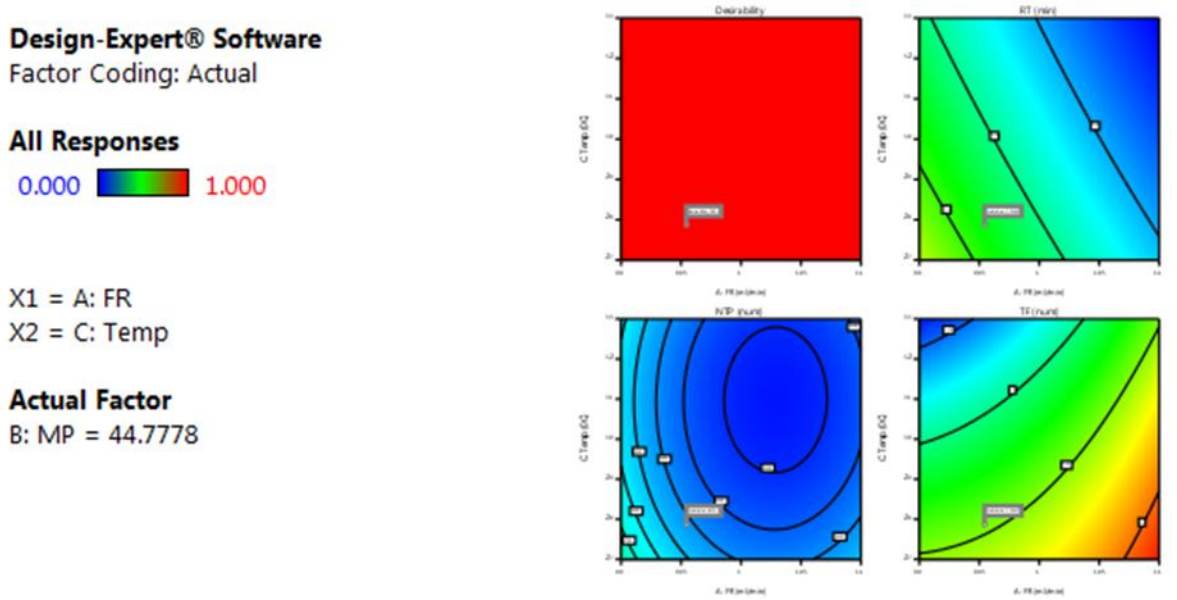
**Table 4 The Responses of Trial**

| | | ***Factor 1*** | ***Factor 2*** | ***Factor 3*** | ***Response 1*** | ***Response 2*** | ***Response 3*** |
|---|---|---|---|---|---|---|---|
| *Std* | *Run* | *A:FR* | *B:MP* | *C:Temp* | *RT1* | *RT2* | *RS* |
| | | *ml/min* | *%* | *0 C* | *min* | *num* | *num* |
| *1* | *4* | *0.9* | *35* | *27* | *3.07* | *8138.4* | *1.3* |
| *2* | *3* | *1.1* | *35* | *27* | *2.49* | *7587.9* | *1.27* |
| *3* | *8* | *0.9* | *45* | *27* | *2.73* | *6866.4* | *1.26* |
| *4* | *12* | *1.1* | *45* | *27* | *2.222* | *6503.1* | *1.32* |
| *5* | *13* | *0.9* | *35* | *33* | *2.738* | *8089.3* | *1.25* |
| *6* | *6* | *1.1* | *35* | *33* | *2.271* | *7324.7* | *1.26* |
| *7* | *14* | *0.9* | *45* | *33* | *2.433* | *6580.7* | *1.13* |
| *8* | *17* | *1.1* | *45* | *33* | *2.02* | *6399.4* | *1.25* |
| *9* | *15* | *0.831821* | *40* | *30* | *2.896* | *7622.5* | *1.24* |
| *10* | *2* | *1.16818* | *40* | *30* | *2.086* | *6832.6* | *1.31* |
| *11* | *11* | *1* | *31.591* | *30* | *2.746* | *8173.9* | *1.28* |
| *12* | *20* | *1* | *48.409* | *30* | *2.259* | *6111* | *1.22* |
| *13* | *19* | *1* | *40* | *24.9546* | *2.689* | *6961.5* | *1.27* |
| *14* | *10* | *1* | *40* | *35.0454* | *2.216* | *6798.8* | *1.18* |
| *15* | *18* | *1* | *40* | *30* | *2.416* | *6585.3* | *1.24* |
| *16* | *9* | *1* | *40* | *30* | *2.418* | *6545.7* | *1.24* |
| *17* | *1* | *1* | *40* | *30* | *2.419* | *6591.9* | *1.24* |
| *18* | *7* | *1* | *40* | *30* | *2.42* | *6580.6* | *1.24* |
| *19* | *5* | *1* | *40* | *30* | *2.421* | *6543.4* | *1.24* |
| *20* | *16* | *1* | *40* | *30* | *2.422* | *6568.5* | *1.24* |

**Table 5 Final Responses**

| *Response* | *Name* | *Units* | *Observations* | *Analysis* | *Minimum* | *Maximum* | *Mean* | *Sd. Dev.* | *Ratio* | *Transform* | *Model* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *R1* | *RT* | *min* | *20* | *Polynomial* | *2.02* | *3.07* | *2.47* | *0.2697* | *1.52* | *None* | *Quadratic* |
| *R2* | *NTP* | *num* | *20* | *Polynomial* | *6111* | *8173.9* | *6970.28* | *626.54* | *1.34* | *None* | *No model chosen* |
| *R3* | *TF* | *num* | *20* | *Polynomial* | *1.13* | *1.32* | *1.25* | *0.0422* | *1.17* | *None* | *Quadratic* |



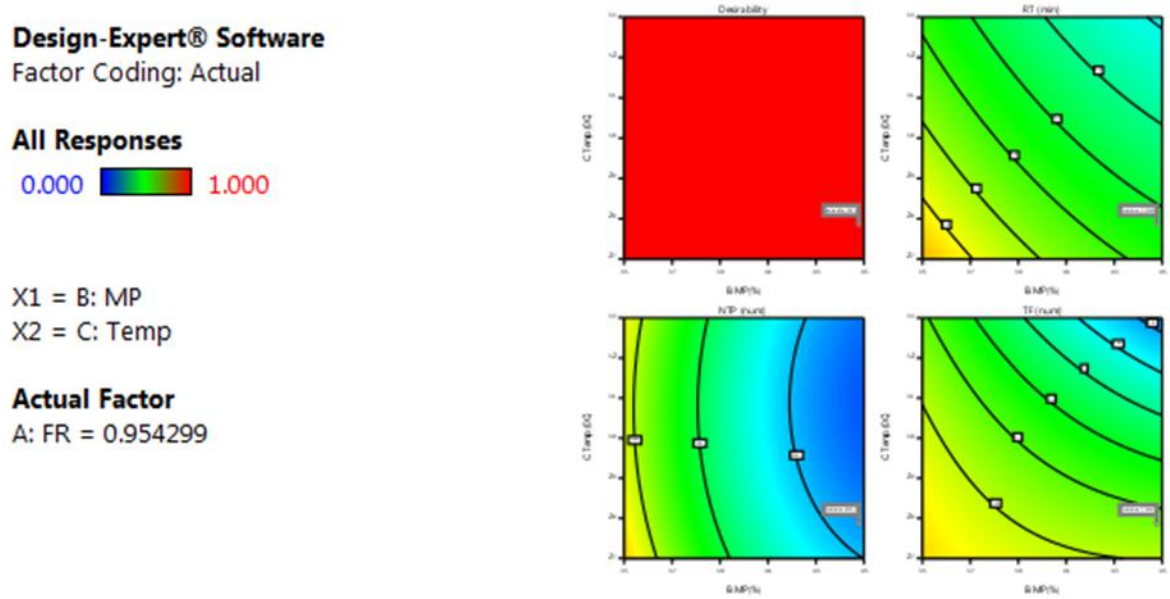



**Figure 4: Multi Contour plots of all Response**

**Validation**

**System Suitability:**

**Table 6: System Suitability**

| | *Tepotinib* | | | |
|---|---|---|---|---|
| ***Injection*** | ***RT*** | ***area*** | ***Plate Count*** | ***Tailing*** |
| ***Injection-1*** | *2.429* | *958577* | *6452* | *1.24* |
| ***Injection-2*** | *2.439* | *959575* | *6471* | *1.23* |
| ***Injection-3*** | *2.442* | *953647* | *6490* | *1.24* |
| ***Injection-4*** | *2.447* | *951367* | *6442* | *1.24* |
| ***Injection-5*** | *2.456* | *959574* | *6592* | *1.23* |
| ***Injection-6*** | *2.465* | *953646* | *6484* | *1.24* |
| ***Mean*** | | *956064* | | |
| ***Std ev*** | | *3597.6* | | |
| ***RSD*** | | *0.4* | | |

Six injections of the standard Tepotinib solution were performed, and the chromatograms that corresponded to each injection were acquired. Observations showed that the percent RSD was less than 2%, the USP tailing was less than 2, and the theoretical plate count surpassed 2,000. Every condition for system appropriateness was satisfied and falls within permissible bounds.

**Linearity:**

Six concentrations ranging from 5.6 to 33.8 µg/ml were prepared and linearity was estimated in a duplicate manner. The linearity equation for Tepotinib was y = 41599x + 9224.1. For the calibration curve over the concentration range, the data have shown a good correlation.

**Table 7: Linearity Data**

| | ***Tepotinib*** |
|---|---|
| ***Concentration (ppm)*** | ****Peak area*** |
| ***0*** | *0* |
| ***5*** | *238420* |
| ***10*** | *473301* |
| ***15*** | *719252* |
| ***20*** | *955561* |
| ***25*** | *1179141* |
| ***30*** | *1406697* |
| ***y :*** | *41599x + 9224.1* |
| ***$R^2$*** | *0.999* |
| ***Slope*** | *6626.1* |
| ***Intercept*** | *340.57* |
| ***LOD*** | *0.02µg/ml* |
| ***LOQ*** | *0.06 µg/ml* |

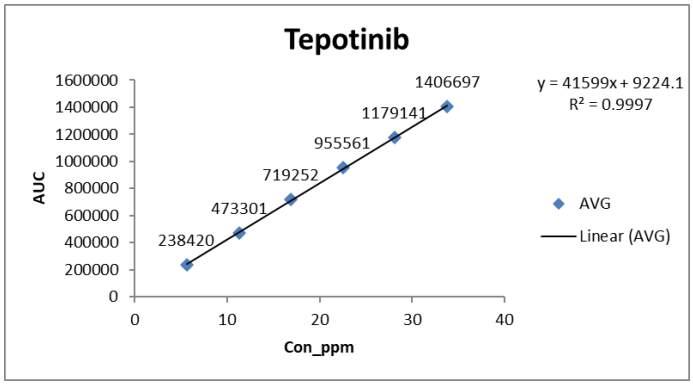



**Figure 5: Calibration Curve of Tepotinib**

**Accuracy:**

Three doses were given at each level, and the mean % recovery was calculated. Tepotinib's recovery rate was observed to be between 99% and 100%, which is within the acceptable ranges

**Table 8: Accuracy Data**

| ***Tepotinib*** | | | | |
|---|---|---|---|---|
| ***% Level*** | ***Added*** | ***recovered*** | ***%Recovery*** | ***Avg %*** |
| *50%* | *11.3* | *11.3* | *100.25* | *100.23* |
| | | *11.3* | *100.11* | |
| | | *11.3* | *100.32* | |
| *100%* | *22.5* | *22.3* | *99.30* | *99.30* |
| | | *22.3* | *99.30* | |
| | | *22.3* | *99.30* | |
| *150%* | *33.8* | *33.9* | *100.39* | *100.29* |
| | | *34.1* | *100.95* | |
| | | *33.6* | *99.54* | |
| ***% recovery*** | | ***99.94*** | | |

## Precision:

**Table 9:** Precision Data

| | *Tepotinib* | |
|---|---|---|
| ***S. No*** | ***Day 1*** | ***Day 2*** |
| ***Injection-1*** | *950636* | *953643* |
| ***Injection-2*** | *958575* | *959557* |
| ***Injection-3*** | *953647* | *954747* |
| ***Injection-4*** | *958574* | *950633* |
| ***Injection-5*** | *953747* | *953746* |
| ***Injection-6*** | *953747* | *953747* |
| ***Mean*** | *954821* | *954346* |
| ***S.D*** | *3142.5* | *2909.1* |
| ***%RSD*** | *0.3* | *0.3* |

## Robustness:

**Table 10: Robustness data for Tepotinib**

| Parameter | Optimized condition | Used condition | Tepotinib Obtained %RSD |
|---|---|---|---|
| Flow rate (±0.1ml/min) | **0.9ml/min** | 0.8ml/min | 0.2 |
| | | 1.0 ml/min | 0.3 |
| MP (5%v/v) | **30:70** | 35:65 | 0.3 |
| | | 25:75 | 0.3 |
| Column temp. (±3$^0$c) | **27$^0$c** | 24 $^0$C | 0.2 |
| | | 30 $^0$C | 0.3 |

## Assay

**Table 11: % Assay Purity Data**

| ***Formulation*** | ***Label claim(mg)*** | ***% Assay**** |
|---|---|---|
| ***Tepmetko*** | ***Tepotinib 225 mg*** | ***99.67 %w/w*** |

## Degradation studies:

**Table 12: Force Degradation Studies of Tepotinib**

| | | Tepotinib | | | |
|---|---|---|---|---|---|
| S.No | Stress Conditions | Optimized area | Peak area | % Degradation | Peak Purity |
| 1 | Acid | *953646* | *928575* | ***0.98*** | Passes |
| 2 | Base | | *905685* | ***5.46*** | Passes |
| 3 | Oxidation | | *925657* | ***5.46*** | Passes |
| 4 | Thermal | | *947575* | ***1.09*** | Passes |
| 5 | Photolytic | | *950364* | ***0.80*** | Passes |
| 6 | Hydrolytic | | *950474* | ***0.78*** | Passes |

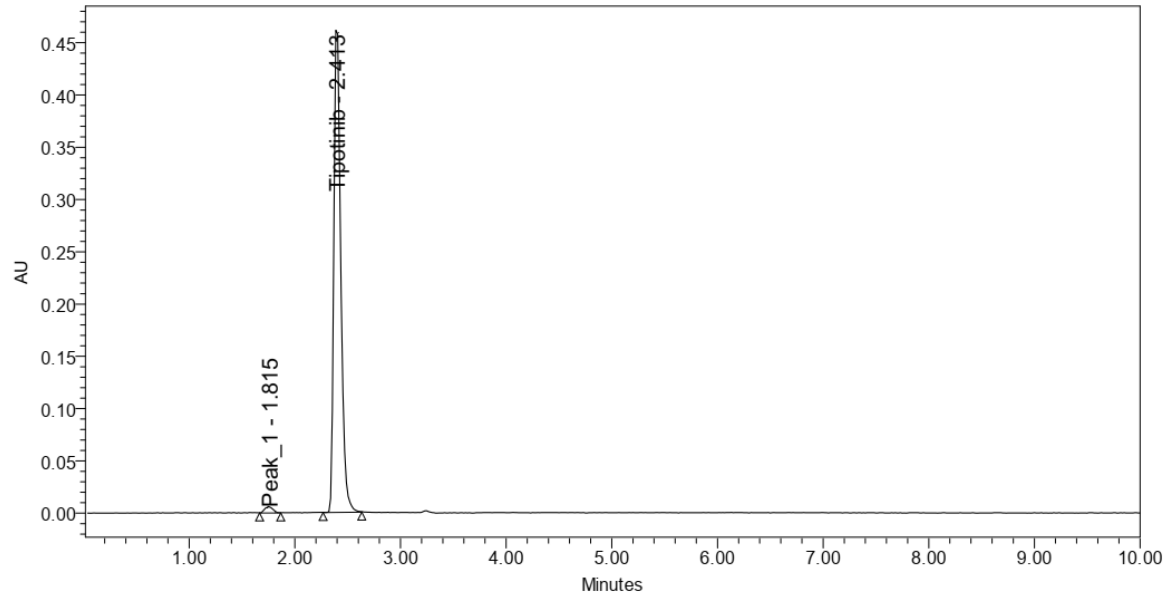



**Figure 6: Acid Chromatogram**

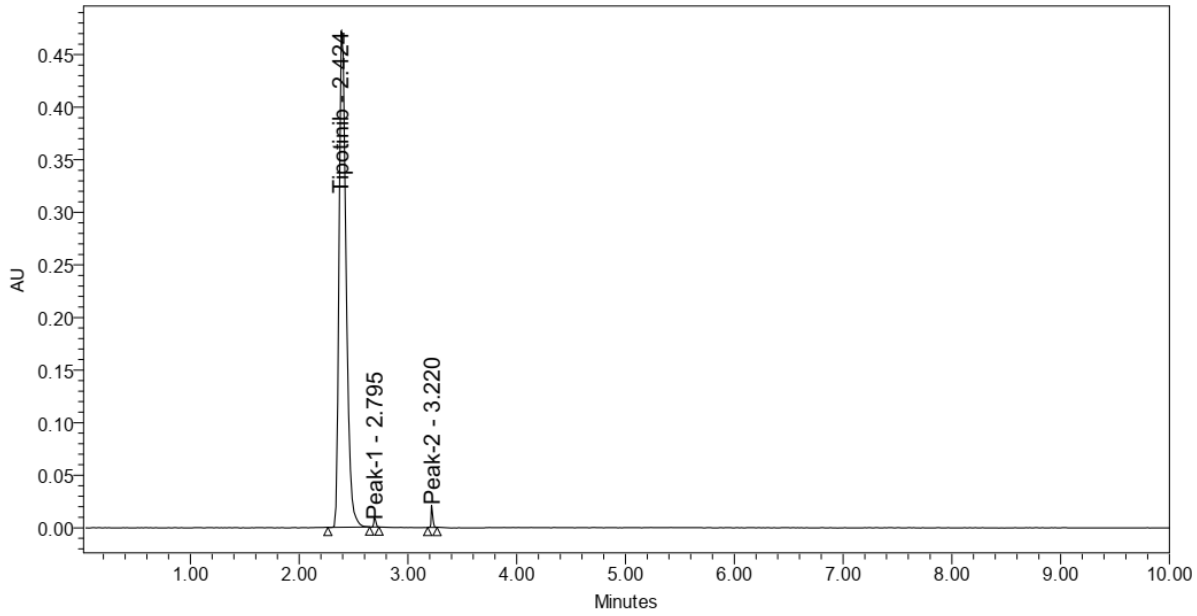



**Figure 7: Base Chromatogram**

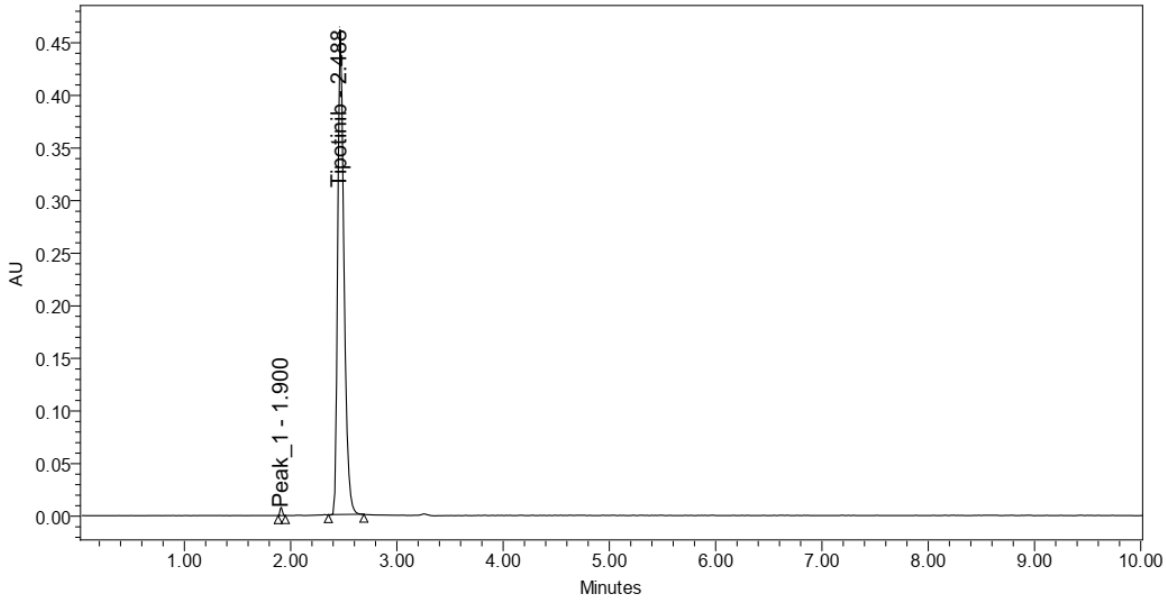



**Figure 8: Oxidative Chromatogram**

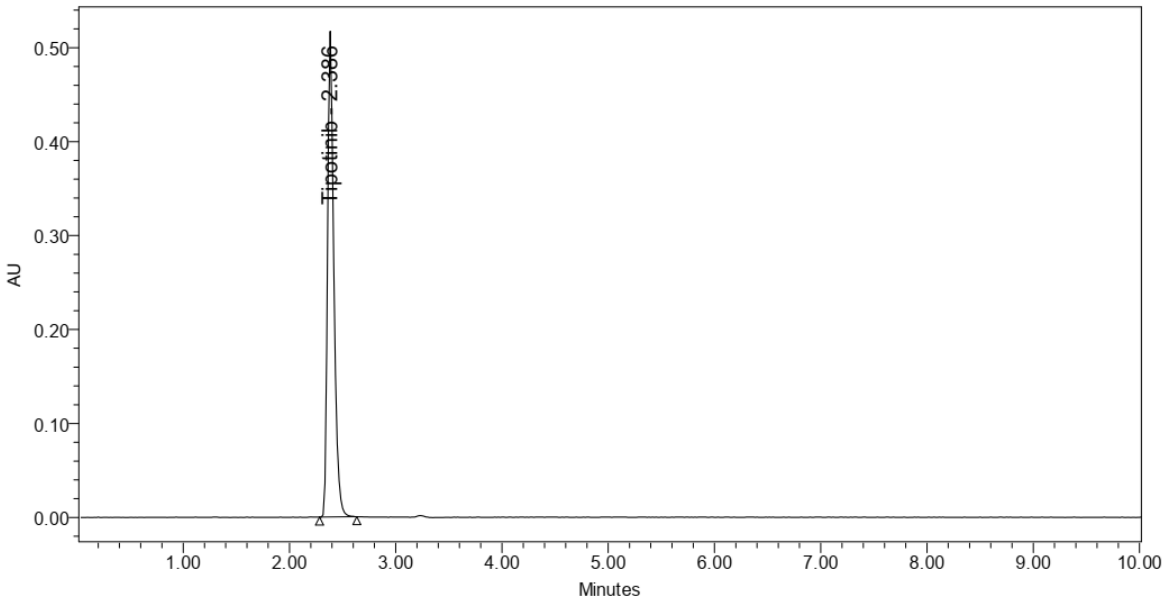



**Figure 9: Thermal Chromatogram**

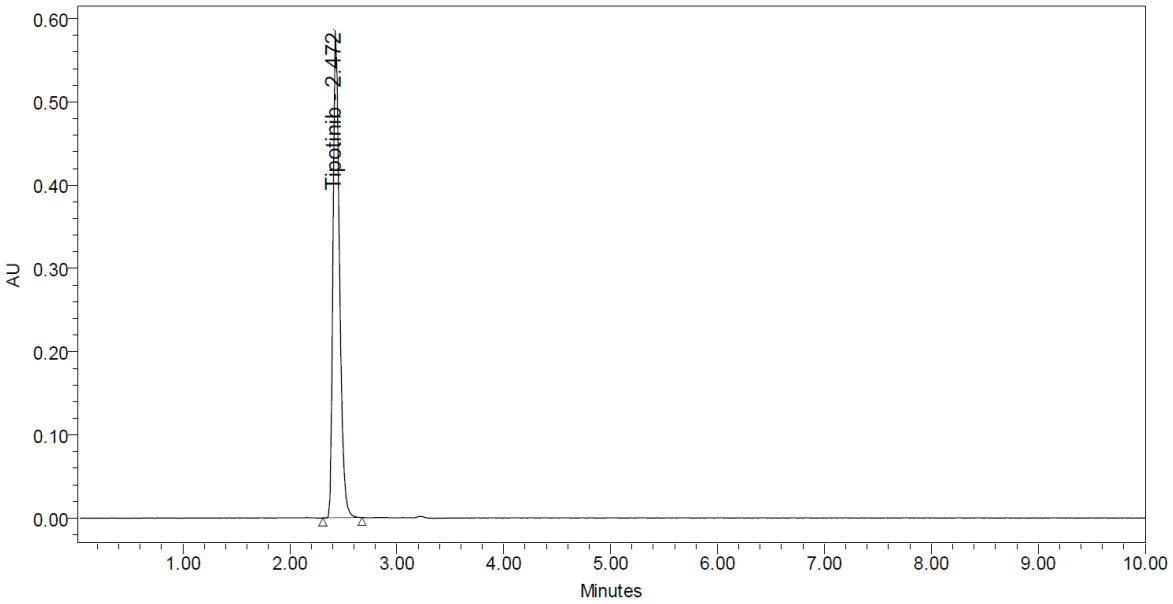



**Figure 10: Photolytic Chromatogram**

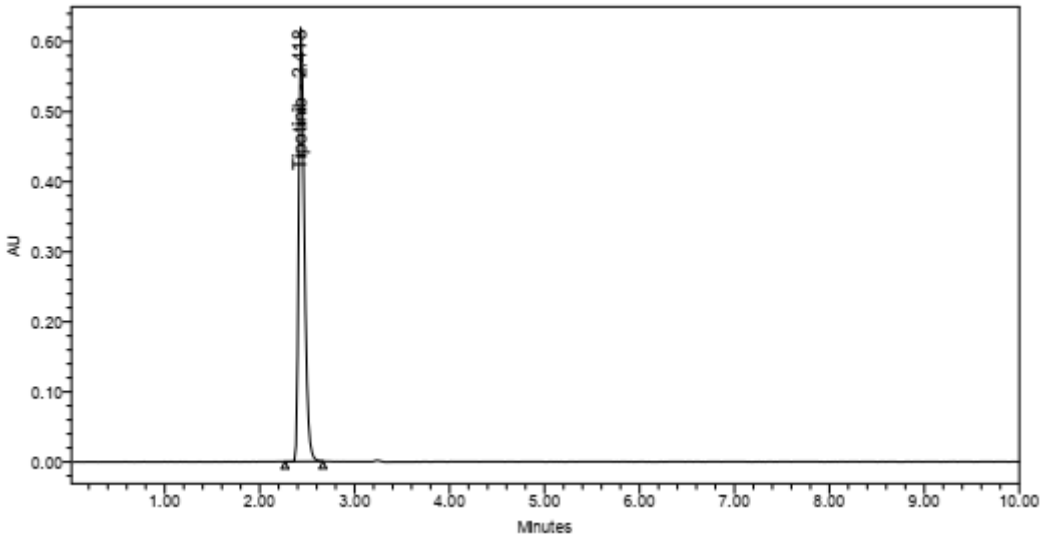



**Figure 11: Water Chromatogram**

**Conclusion:**

By applying QbD principles to HPLC method development, we have ensured that the method is robust, reliable, and capable of producing consistent results. This approach has also facilitated the identification of critical parameters and their interactions, enabling the development of a control strategy to maintain the quality of the method." Based on the results of this QbD HPLC study, the following conclusions can be drawn: - The critical process parameters (CPPs) that significantly affect the critical quality attributes (CQAs) of the HPLC method have been identified. The QbD approach has enabled a systematic and scientific understanding of the HPLC method, ensuring that the final product meets the desired quality attributes a simple, Accurate, precise method was developed for the estimation of the Tepotinib in bulk and pharmaceutical dosage form. Retention time of Tepotinib was found to be 2.422 min. %RSD of the Tepotinib were and found to be 0.4 and 0.3 respectively.

%Recovery was Obtained as 99.94% for Tepotinib. LOD, LOQ values were obtained from regression equations of Tepotinib were 0.02ppm, 0.06 ppm respectively. Regression equation of Tepotinib is y = 41599x + 9224.1.

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