# Development And Validation of Stability Indicating RP-HPLC Method For the Estimation of Tepotinib in Bulk and Formulation

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***Abstract-*** Objective: The present study is to develop a highly suitable simple, specific, rapid and validated stability-indicating reverse-phase high performance liquid chromatography method for the estimation of tepotinib in bulk and pharmaceutical formulation as per ICH guidelines. Method: The chromatographic separation of components was achieved on a Phenomenox Kinetex XB-C18(150×4.6 mm, 5µ) column. The flow rate was 1.0 ml/ min and the absorbance were noted at 272 nm. The procedures and abilities of the method were evaluated against the criteria for linearity, precision, accuracy, system suitability, specificity, and robustness according to the ICH guidelines. Results: The retention time for Tepotinib was 3.4 min. The calibration plot was linear in the range 5-25µg/ml and (r2=0.9993) and the % mean recoveries for accuracy and precision of tepotinib were in the range (%Relative Standard Deviation< 2). The Limit of Detection and Limit of Quantification were determined to be 0.429 µg/ml and 1.3 µg/ml respectively.

***Keywords:*** Tepotinib, Validation, Stability-indicating, Reverse phase high performance liquid chromatography, ICH guidelines, Retention time, 272nm.

## Ⅰ. INTRODUCTION

For the treatment of metastatic non-small cell lung cancer in individuals with MET exon 14 skipping mutations, tepotinib is an oral tyrosine kinase inhibitor [1]. Tepotinib is known by its chemical name 3-[1-[3-[5-[(1-methyLpiperidin-4-yl) methoxy] pyrimidin-2-yl] phenyl] methyl]-6-oxopyridazin-3-yl] benzonitrile. It has a 492g/mol molecular weight. Its chemical structure is $C_{29}H_{28}N_6O_2$. Tepotinib structure is shown in Figure 1. Tepotinib is a MET tyrosine kinase inhibitor used to treat various solid tumours that overexpress MET. Tepotinib preferentially binds to MET tyrosine kinase and blocks MET signalling pathways, which may cause tumour cells that overexpress this kinase to undergo apoptosis [2]. Tepotinib is soluble in acetonitrile, water, methanol, and ethanol. As a result, methanol was used as a diluent for the job.

From the literature survey, it was found that few methods are available for the estimation of Tepotinib by RP-HPLC methods [3,4], spectrofluorometric methods [5]. The aim of the study is to develop and validate stability-indicating RP-HPLC method for the estimation of Tepotinib in bulk and pharmaceutical formulation as per ICH guidelines [9].

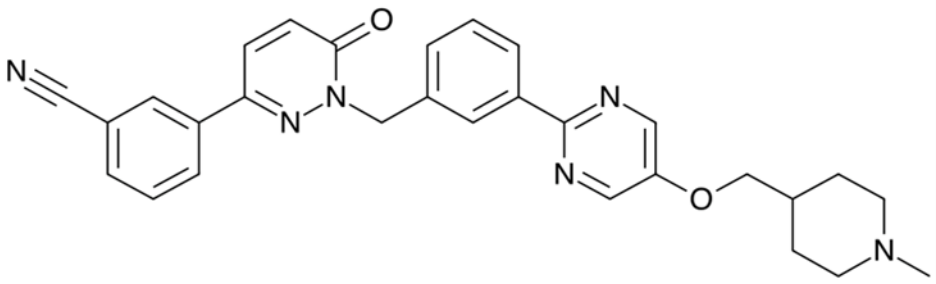


**Fig 1: Structure of Tepotinib**

## Ⅱ. MATERIALS AND METHODS

*Chemicals and reagents:*

API of Tepotinib as reference standard was procured from Spectrum Pharma Research Solutions Pvt Ltd, Hyderabad. Tepmetko tablets were purchased from India Mart app.

HPLC grade methanol and water and acetonitrile were purchased from Merck Pvt. Ltd., Mumbai.

*Instrumentation*

Analytical balance (Shimadzu AY220), Ultra Sonicator (Oscar Microclean 103), High-Performance Liquid Chromatography (Agilent 1260 Infinity II auto sampler)

*Optimized Chromatographic conditions [8]:*

Optimized chromatographic conditions are mentioned in the table 1.

**Table 1: Optimized conditions**

| Sr. No. | Parameters | Optimized Conditions |
|---|---|---|
| 1 | Column | Phenomenex Kinetex XB-C18 ((150×4.6mm, 5µ) |
| 2 | Column Temperature | 30ºC |
| 3 | Mobile phase | Acetonitrile: Water |
| 4 | Mobile Phase Ratio | 80:20 V/V |
| 5 | Diluents | Methanol |
| 6 | Flow Rate | 1ml/min |
| 7 | Injection Volume | 10µl |
| 8 | Wavelength | 272nm |
| 9 | Retention Time | 3.4 min |
| 10 | Run Time | 10 min |

*Preparation of Standard stock solution:*

10 mg of tepotinib was weighed and transferred into 10 ml of volumetric flask, 5ml of the methanol was added to the tepotinib and sonicated for 10 min. the volume was made up to the mark with methanol. From the above solution take 1ml of solution and transferred to 10ml volumetric flask and volume was made up to the mark with methanol [8].

*Preparation of Sample solution:*

Tablet powder equivalent to 10 mg of tepotinib was taken into 10 ml volumetric flask 5ml of methanol was added and sonicated for 10 min and volume was made up to the mark with the same. From the above solution 0.1ml of sample transferred to 10 ml of volumetric flask. Add 5 ml of methanol sonicated for 10min and volume was made up to the mark with the same [8,9].

*Selection of Wavelength:*

A solution of tepotinib in the mobile phase was prepared and scanned in the UV range 200-400nm in UV -VIS spectrophotometer, and $\lambda_{max}$ was selected as 272 nm from UV spectrum [8,9].

**Forced Degradation Studies:**

Forced degradation studies were performed to determine the degradation characteristics of the drug and stability indicating properties of the strategy. All forced degradation studies

were analyzed at 10µg/ml concentration level. Acid degradation was carried out by treating drug solution with 1ml of 0.1N HCl, alkaline degradation was conducted by treating with 1ml of 1N NaOH and oxidative degradation was carried out by treating with 1ml of 10% $H_2O_2$, after keeping at room temperature for 10 min, the solution was neutralized and diluted with diluents. Degradation with dry heat was carried out by 10mg of drug was kept into hot air oven at 80ºC for 5 hrs. Sample solution (Conc. of Tepotinib- 10µg/ml) of tepotinib was filtered through 0.45µm syringe filter and injected into chromatographic system to measure the peak area [17].

## Ⅲ. METHOD VALIDATION

*1) Linearity and range:*

The linearity of the method was performed by using different linearity solutions in diluent. For the linearity studies of tepotinib the concentration was determined over the range 5-25 µg/ml. Calibration curve plotted by taking the peak area verses concentration in µg/ml. From the calibration curve the values of coefficient of regression, slope and Y-intercept was calculated [9,10].

*2) Accuracy:*

To perform the accuracy of the present method, recovery studies was performed by standard addition method. In this method, 80, 100, 120% of three different levels of pure drug were added to the previously analysed sample solution in 10ml of volumetric flask and diluted upto the mark with methanol. Solutions were filtered through 0.45µm syringe filter and injected in an HPLC system to measure the peak area. Accuracy was calculated as recovery values of the tepotinib [9,10].

*3) Precision:*

Precision was estimated by repeatability studies. By injecting the six samples of tepotinib solution (µg/ml) on the same day and a different day. The precision of the method was calculated as %RSD of the response [11].

*4) Robustness:*

Deliberate changes in the chromatographic conditions such as flow rate, detection wavelength etc. After each change, assay results were checked by injecting the tepotinib solution into chromatographic system, and results were compared with the original results which were taken under original chromatographic conditions [11].

*5) System suitability:*

System suitability parameters such as capacity factor, tailing factor, number of theoretical plates, and resolution of peak were carried by injecting a blank mobile phase followed by tepotinib solution [12-14]. (Table 2).

**Table 2: System suitability parameters**

| Parameter | Values |
|---|---|
| Wavelength | 272nm |
| Retention Time | 3.4min |
| Theoretical Plates | 2390 |
| Tailing Factor | 1.65 |
| Resolution | 1.7 |

*6) LOD and LOQ:*

Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under stated chromatographic conditions. The limit of quantification can be determined with acceptable accuracy and precision. LOD can be calculated by using formula $LOD = 3.3 \times S.D \div S$ and LOQ can be calculated by $LOQ = 10 \times S.D \div S$, where, S.D is the standard deviation of response and S is the slope of the calibration curve [12-14].

## Ⅳ. RESULTS

*Physical characterization of Tepotinib:*

Tepotinib powder was analysed for the physical properties like colour, solubility. The drug was also scanned with the help of UV spectrophotometer. The absorption maxima for Tepotinib were found to be 272nm using methanol as solvent. Figure 2 gives the UV spectrum of Tepotinib. Figure 3 indicates the obtained chromatographic peak of tepotinib solution.

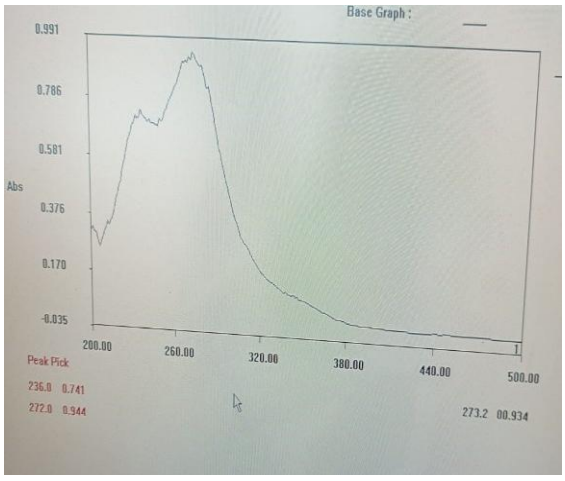


**Fig 2: UV spectrum of Tepotinib**

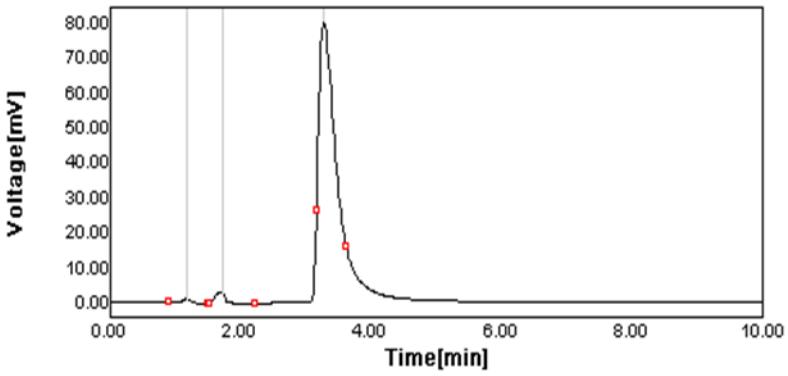


**Fig 3: Chromatogram of Tepotinib solution**

*Method Validation:*

*Linearity:*

The absorbance is proportional to the concentration and linear (Table 3). The value of correlation coefficient ($r^2$) was 0.9993 which is well within acceptance limit ($r^2$<1). The Calibration curve graph is shown in Figure 3. Figure 5 shows linearity overlay of different concentrations of tepotinib.

**Table 3: Linearity results**

| Concentration (µg/ml) | Peak area |
|---|---|
| 5 | 329 |
| 10 | 725 |
| 15 | 1076 |
| 20 | 1498 |
| 25 | 1853 |

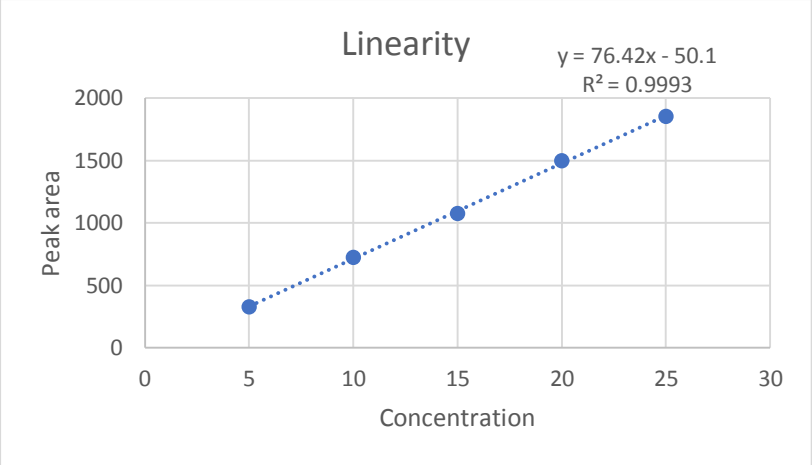


**Fig 4: Calibration curve of Tepotinib**

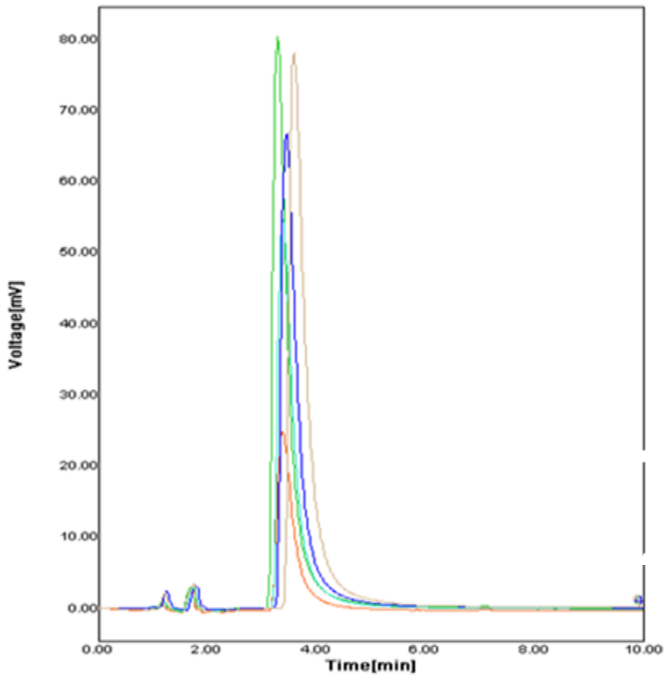


**Fig 5: Linearity overlay of Tepotinib**

*Range:*

The range of analytical method was selecte om 5-25µg/ml.

*Accuracy:*

The percentage recoveries of the result ndicate that the recoveries are well within acceptance range (RSD<2), therefore method is accurate. Table 4 indicates the % recovery studies of tepotinib.

**Table 4: Accuracy results**

| Sr. No | % Level | Amount spiked | Amount recovered | % Recovery |
|---|---|---|---|---|
| 1 | 80 | 18 | 18.3 | 101.7 |
| 2 | 100 | 20 | 20.04 | 100.2 |
| 3 | 120 | 22 | 21.83 | 99.26 |

*Precision:*

The precision was calculated by repeatability. The following Table 5 gives the results of precision.

**Table 5: Precision results**

| Readings | Conc. | Peak Area | | Statistical Analysis | |
|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 1 | Day 2 |
| 1 | | 1123 | 1119 | Mean=1114.66 | Mean=1115 |
| 2 | | 1106 | 1100 | SD=10.269 | SD=9.591 |
| 3 | 15 (µg/ml) | 1128 | 1126 | %RSD=0.921 | %RSD=0.860 |
| 4 | | 1118 | 1108 | | |
| 5 | | 1112 | 1115 | | |
| 6 | | 1101 | 1122 | | |

*Values given in the table are mean ±SD, n= 6 responses, %RSD: Relative standard deviation

*Limit of Detection (LOD):*

Limit of Detection was determined to be 0.429µg/ml.

*Limit of Quantification (LOQ):*

Limit of Quantification was determined to be 1.3µg/ml.

*Robustness:*

Table 6 gives the robustness results of the tepotinib solution.

**Table 6: Robustness results** (Concentration. of Tepotinib- 15µg/ml)

| Sr.no. | Parameter | Optimized | Used | Peak Area | % Recovery |
|---|---|---|---|---|---|
| 1 | Flow rate(±0.1ml) | 1.0ml/min | 0.9ml/min | 1119 | 99/6 |
| | | | 1.1ml/min | 1110 | 98 |
| 2 | Detection wavelength(±1nm) | 272nm | 271nm | 1126 | 100.2 |
| | | | 272nm | 1118 | 99.5 |

*Forced Degradation Studies:*

Tepotinib was found more sensitive to base degradation (Figure 7); the assay value was decreased to 85.32%. It had been conjointly susceptible to acidic, oxidative and dry heat degradation yielding % degradation values of 6.70 (Figure 6), 6.74 (Figure 8) and 3.03 (Figure 9). Table 7 gives the results obtained from forced degradation studies.

**Table 7: Forced Degradation Studies**

| Parameters | Area | Assay | % Degradation |
|---|---|---|---|
| Acid degradation | 3185 | 93.21 | 6.70 |
| Base degradation | 4763 | 85.32 | 14.59 |
| Oxidative degradation | 3183 | 93.17 | 6.74 |
| Degradation with Dry Heat | 3390 | 96.89 | 3.03 |

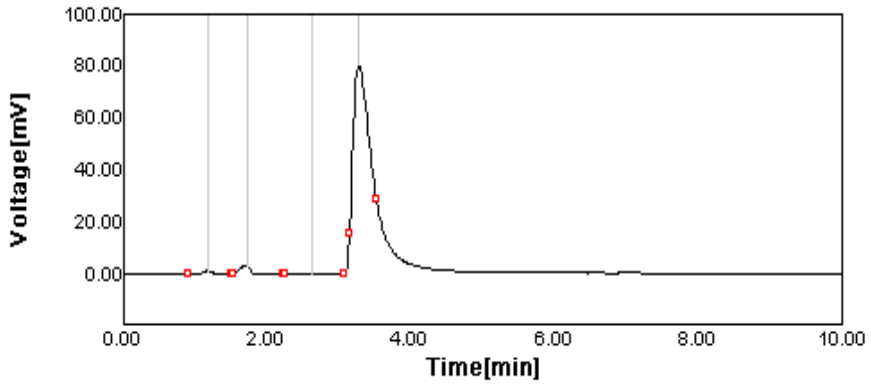


**Fig 6: Chromatogram of Acid Degradation**

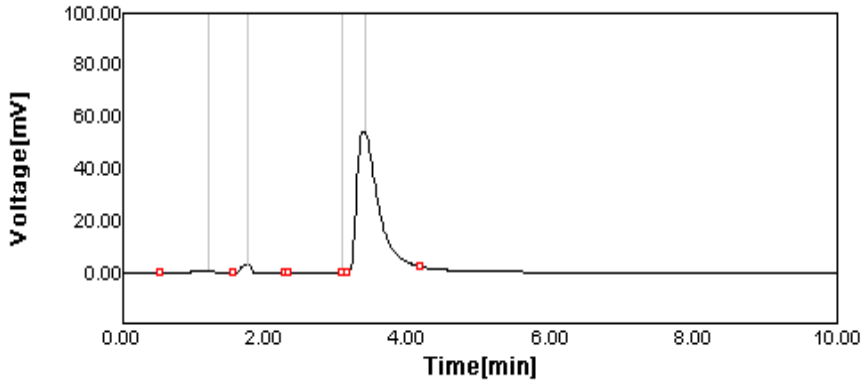

**Fig 7: Chromatogram of Base Degradation**

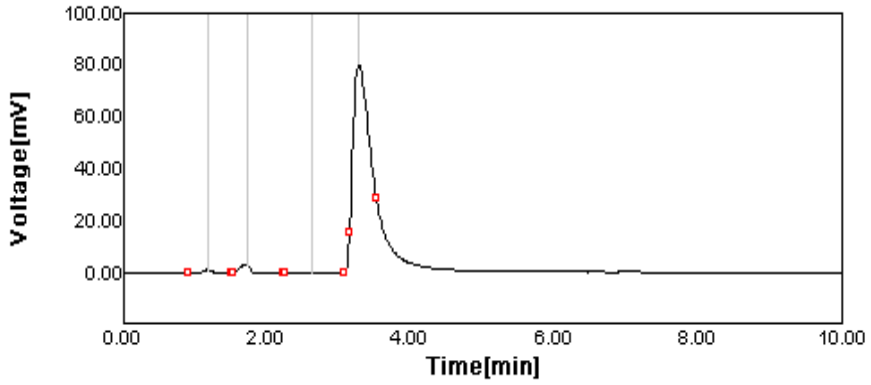


**Fig 8: Chromatogram of Oxidative Degradation**

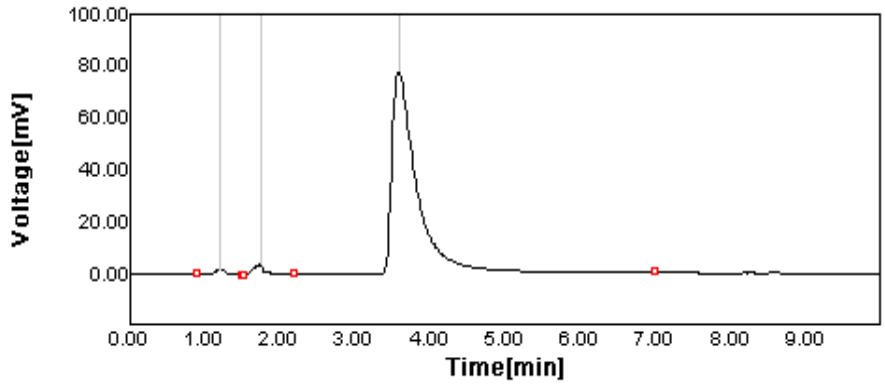


**Fig 9: Chromatogram of Degradation with Dry heat**

*Specificity:*

The comparison of the information of the drug solution before spiking and spiked drug solution discovered that there was no significant interference of blank with the recovery of Tepotinib, hence the method was specific. (Table 8). Figure 10 gives information about chromatogram of mixture of blank, working solution, drug product solution.

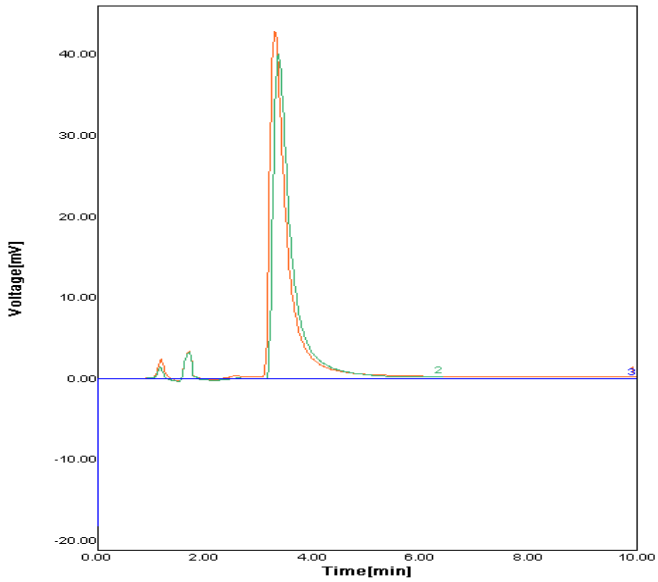


**Fig 10: Chromatogram of mixture Blank, Working solution, Drug Product solution**

**Table 8: Specificity results**

| Sample | Retention time (min) | Area | % Assay |
|---|---|---|---|
| Blank | – | – | – |
| Working Solution | 3.4 | 1094 | – |
| Drug product | 3.31 | 1190 | 99.90 |

*Assay:*

The developed high performance liquid chromatography method was applied to the determination of the Tepotinib content in the marketed formulation. Average percent assay of Tepotinib tablet was found to be 98%.

**Summary:**

Table 9 gives summary of validation parameters.

**Table 9: Summary of Validation Parameter**

| Parameter | Values |
|---|---|
| Maximum wavelength (nm) | 272 |
| Range (µg/ml) | 5-25 |
| Regression equation | Y= 72.46x-50.1 |

| | |
|---|---|
| Precision (% RSD) | Intra-day-0.9213244 |
| | Inter-day-0.860239 |
| LOD (µg/ml) | 0.429 |
| LOQ (µg/ml) | 1.3 |
| Robustness | Robust |

## V. DISCUSSION

The closeness to the higher theoretical plates and low tailing factor indicates that the system is efficient.

The calibration curve for different concentration of tepotinib were plotted. From the calibration curve data regression equation was found to be Y=76.42-50.1 and the correlation coefficient was noted as $R^2$= 0.9993. Linearity was found to be within the concentration 5-25µg/ml.

The accuracy of the method was calculated by percentage recovery studies. Percentage recovery was found to be in the range 99.26-101.7%. The results were within the limit. Hence, method was found to be accurate.

The precision of the method was studied by repeatability. %RSD for intra-day precision and inter-day precision was found to be 0. 92and 0.86 respectively.

The robustness of the method was performed by deliberate change in the detection wavelength and flow rate. % Recoveries was found to be in the limits. Hence, the proposed method was found to be robust.

On the basis of mobile phase, mobile phase ratios, flow rate, wavelength, limits, accuracy and application of the HPLC methods, a comparison of previously published work and recently performed HPLC method was carried out. The details are enlisted in the table 10.

When compared to formerly developed work and other related published literature, the recent method, which uses Acetonitrile and water (80:20V/V), 1ml/min flow rate, 272 nm detection wavelength, is shown to more accurate, precise, economic, cost effective. According to the ICH guidelines all the parameters were within the limits, hence the established method was validated.

**Table 10: Comparison table**

| Sr no. | Mobile Phase and ratio | Flow rate | Wavelength (nm) | Column | Limitations | Reference |
|---|---|---|---|---|---|---|
| 1 | Buffer: Acetonitrile (50:50V/V) | 1ml/ min | 254 | Agilent eclipse C18 column (150x4.6mm,3.5µ) | Use of buffer as mobile | 3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | phase, less sensitive | |
| 2 | Acetonitrile: 0.1% OPA (50:50V/V) | 1ml/min | 310 | Ascentis (150 mm× 4.6 mm, 2.7 µm) | Use of buffer as mobile phase, less sensitive, expensive | 4 |
| 3 | Acetonitrile: 0.1% OPA (50:50V/V) | 1ml/min | +ve mode of electro spray ionization by using MS | Waters symmetry C18 column (150x4.6mm,3.5µ) | Use of buffer as mobile phase, expensive due use of MS detection, | 6 |
| 4 | 0.02 M potassium dihydrogen phosphate–acetonitrile (7:3 V/V) | 1ml/min | 265 | C8 column (250 mm×4.6 mm, 5µm) | Use of buffer as mobile phase, expensive | 19 |

## VI. CONCLUSION

Thus, it can be concluded that the method developed in the present investigation was linear, simple, rapid accurate, precise, specific, sensitive and precise. The method exhibits simplicity in terms of short analysis time, gradient mode of elution of mobile phase, effective and clear resolution with low LOD and LOQ values. This method proposed method was developed and validated as per the ICH guidelines.

## VII. ACKNOWLEDGEMENT

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## VIII. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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