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# DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE DETERMINATION OF TIPRANAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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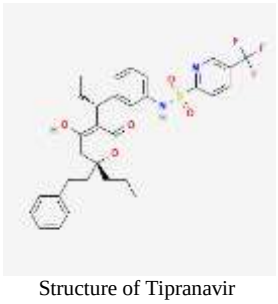
*Abstract :*

A new RP-HPLC for the simultaneous estimation of Tipranavir was developed by optimizing the chromatographic conditions using Inertsil ODS C18 column (4.6×150mm) 5µ and the flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer ($KH_2PO_4$and $K_2HPO_4$) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 265nm.the instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 1.988 mins .The % purity of Tipranavir was found to be 99.24% and. The system suitability parameters for Tipranavir such as theoretical plates and tailing factor were found to be2689, 1.4 and the resolution was found to be 8.86. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Tipranavir was found in concentration range of 10µg-60µg and correlation coefficient ($r^2$) was found to be 0.999, recovery was found to be 99.56%,%RSD for repeatability was 0.5, % RSD for intermediate precision was 0.6 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 1.97 and LOQ value was 1.97. Hence the suggested RP-HPLC method can be used for routine analysis of Tipranavir in API and Pharmaceutical dosage form.

***Key words : tipranavir,RP-HPLC,* correlation coefficient.**

## I. INTRODUCTION

Tipranavir, or tipranavir disodium, is a nonpeptidic protease inhibitor (PI) manufactured by Boehringer-Ingelheim under the trade name Aptivus. It is administered with ritonavir in combination therapy to treat HIV infection and is given as 250 mg capsules together with 200 mg of ritonavir twice daily.



Structure of Tipranavir

Spectrophotometer, HPLC and UPLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new hplc development for the simultaneous estimation of Tipranavir in pharmaceutical dosage form.Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method and spectroscopic method for the simultaneous analysis of Tipranavir. The developed method was been validated according to ICH guidelines.

**METHOD DEVELOPMENT:**

Method development for simultaneous estimation of Tipranavir in Pharmaceutical dosage forms includes the following steps:

1. Selection of Detection wavelength:

10 mg of Tipranavir was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained.

2. Selection of column:

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Agilent C18 (4.6 x 250mm, 5µm].

3. Selection of mobile phase:

Methanol: ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

4. Selection of flow rate:

Flow rate selected was 1ml/min Flow rate is selected based on Retention time, Column back pressure, Peak symmetry.Separation of impurities.

5.Preparations and procedures:

a)Preparation of mobile phase:

A mixture of Methanol 700ml (70%), 300 mL of ACN (30%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

b)Preparation of the individual Tipranavir standard preparation:

10mg of Tipranavir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of methanol is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluent .
Further 1.0 ml from the above stock solution is pipetted into a 100 ml volumetric flask and was diluted up to the mark with diluent.

c)Preparation of Sample Solution :

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Tipranavir (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of s is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a1ml volumetric flask and diluted upto the mark with diluant.

PROCEDURE:

20µL of the standard, sample are injected into the chromatographic system and the areas for Tipranavir peaks are measured and the %Assay are calculated by using the formulae.

SYSTEM SUITABILITY:

Tailing factor for the peaks due to Tipranavir in Standard solution should not be more than 2.0. Theoretical plates for the Tipranavir peaks in Standard solution should not be less than 2000

***Assay calculation:***

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

P = Percentage purity of working standard
Lc = label claim of drug in mg/ml.

ANALYTICAL METHOD VALIDATION:

1. ACCURACY:

Preparation of standard solution (Tipranavir):

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

Preparation of Standard stock solution:

10mg of Tipranavir was accurately weighed and transferred into a 10 ml clean dry volumetric flask; about 10 ml of was added and sonicate to dissolve it completely. The volume was made up to the mark with the same solvent. (Stock solution) .Further 0.1 ml was pipette out from the above stock solutions into a 10ml volumetric flask and diluted up to the mark with to give the concentration of 100 µg/ml .

Preparation of Sample solutions:
Preparation of 50% solution (90µg/ml of Tipranavir):
From the above stock solution take 9 ml into 10 ml dry volumetric flask, make up to the mark with .
Preparation of 100% solution (120 µg/ml of Tipranavir):
From the above stock solution take 12 ml dry volumetric flask, make up to the mark with
Preparation of 150% solution (180µg/ml of Tipranavir ):
From the above stock solution take 18 ml into 10 ml dry volumetric flask, make up to the mark with . These solutions were filtered through 0.45µ membrane and then each concentration; three replicate injections were made under the optimized condition,
The standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions were injected. The amount found and amount added for Tipranavir recovery and mean recovery values were calculated.

2.PRECISION:

A)Repeatability Procedure: The standard stock solution was injected for five times and the areas for all five injections in HPLC were measured. The %RSD for the area of five replicate injections was found to be within the specified limits.

B) Intermediate Precision (Ruggedness):
To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different column of same dimensions.
3.SPECIFICITY:
The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.
4.LOD:
LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

*Formula:*

$$LOD = 3.3 \times \frac{\sigma}{S}$$

σ - Standard deviation (SD)
S – Slope

5.LOQ:
LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

*Formula:*

LOQ=10 σ/Slope

Where
σ - Standard deviation
S – Slope
6. Linearity
Preparation of sample stock solution:
About 10 mg of Tipranavir samples was weighed in to 10ml volumetric flask, it was dissolved with diluents and the volume was made up to the mark with same (1000µg/ml of Tipranavir).

Preparation of Level – I (20µg/ml of Tipranavir )

0.2 ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent .

Preparation of Level–II (40µg/ml of Tipranavir )

0.4ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–III (60µg/ml of Tipranavir )

0.6 ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–IV (80µg/ml of Tipranavir )

0.8ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level–(100µg/ml of Tipranavir )
1ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

.Preparation of Level–(120µg/ml of Tipranavir )
1.2ml of stock solution had taken in 10ml of volumetric flask diluted up to the mark with diluent.

10µl of each level were injected into the system and recorded the peak response.

PROCEDURE:

Each level solution was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated.

The linearity of the method was demonstrated over the concentration range of 10-100µg / ml. Aliquots of five levels were prepared from sample solution and labeled as solution 1, 2, 3, 4 and 5 respectively. The solutions were injected in to HPLC system as per test procedure. A calibration curve was plotted for concentration v/s peak area.

1.Range:

Based on precision, linearity and accuracy data it can be concluded that the assaymethod is precise, linear and accurate in the range of **1µg-5µg Tipranavir**

2. Robustness:

As a part of robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.8ml/min to 1.2 ml/min. Standard solution 60ppm of Tipranavir was prepared and analyzed using the varied flow rates along with method flow rate.

b) The organic composition in the mobile phase was varied from 70% standard solution 60 µg/ml Tipranavir was prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method.

3. System suitability:

10 mg of Tipranavir working standard was accuratelyweighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of s and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further 10 ml of Tipranavir out from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

## II. RESULTS AND DISCUSSION

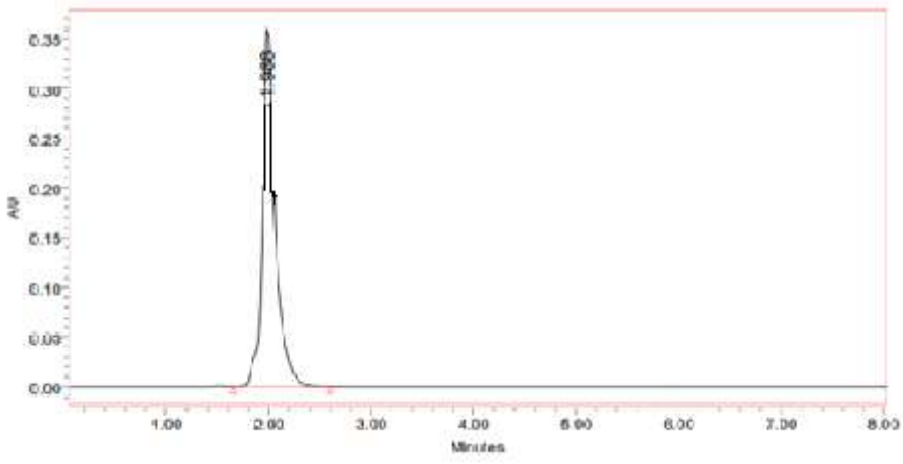
Literature reveals that there are no analytical methods reported for the simultaneous estimation of Tipranavir by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Tipranavir in pharmaceutical dosage form.

Method Development:

Optimized chromatographic conditions for simultaneous estimations of tipranavir by RP-HPLC method

| | | |
|---|---|---|
| Column | : | Inertsil ODS 4.6*150 , 5µ |
| Column temperature | : | Ambient |
| Wavelength | : | 265 nm |
| Mobile phase ratio | : | 70:30 methanol : phosphate buffer $p^H$ 3 |
| Flow rate | : | 1 ml/min |
| Auto sampler temperature | : | Ambient |
| Injection volume | : | 10µl |
| Run time | : | 8.0 minutes |

**Fig no -1 showing Optimized Chromatogarphic method**




Assay calculation for tipranavir:

The assay study was performed for the tipranavir. Each three injections of sample and standard were injected into chromatographic system and results are tabulated in **Table.No.1**.

**Table.No.01. Showing assay results**

| | Peak name | RT | Area | Height | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | tipranavir | 1.989 | 3162617 | 358347 | 1496 | 1.2 |
| 2 | tipranavir | 1.989 | 3194044 | 361853 | 1489 | 1.2 |
| 3 | tipranavir | 1.988 | 3169773 | 361297 | 1483 | 1.2 |
| 4 | tipranavir | 1.988 | 3193022 | 358621 | 1490 | 1.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | tipranavir | 1.988 | 3205507 | 361678 | 1476 | 1.2 |
| 6 | tipranavir | 1.987 | 3194053 | 361472 | 1496 | 1.2 |
| mean | | 2.0 | 3186144 | 361740 | | |
| Std.dev | | 0.0 | 1640.4 | | | |
| %RSD | | 0.0 | 0.5 | | | |

The retention time of tipranavir is found to be 1.98 mins. The system suitability parameters for tipranavir such as theoretical plates and tailing factor was found to be 1.2 Resolution was 6.0 The % purity tipranavir pharmaceutical dosage form was found to be 99.24 and 101.27% respectively.

VALIDATION REPORT:

1. Specificity:

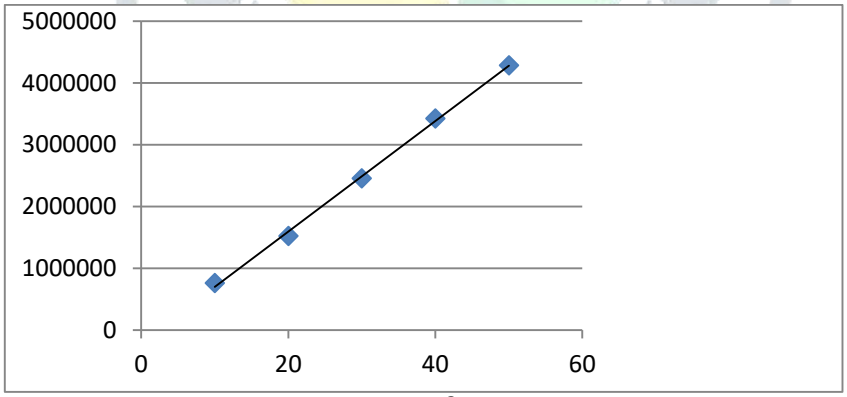
The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The specificity test was performed for tipranavir . It was found that there was no interference of impurities in retention time of analytical peak.

.2. Linearity :

The linearity study was performed for the concentration of 10 ppm to 60 ppm level. Each level is injected into chromatographic system. The area of each level was used for calculation of correlation coefficient and results are tabulated also Calibration graph for tipranavir is shown below.

**Table no 2 Linearity Results for tipranavir :**

| S.No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 10 ppm | 759201 |
| 2 | II | 20 ppm | 1522876 |
| 3 | III | 30 ppm | 2284847 |
| 4 | IV | 40 ppm | 3424253 |
| 5 | V | 50 ppm | 4285917 |
| Correlation Coefficient | | | 0.999 |




**Tipranavir $r^2$ = 0.999**

3.Accuracy :

The accuracy study was performed for 50%, 100% and 150 % for tipranavir

.Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery and results are tabulated in **table no-3**.

**Table.No.3. Showing accuracy results for tipranavir**

| %Concentration (at specification level) | Average area | Amount added (mg) | Amount found (mg) | % Recovery | Mean recovery |
|---|---|---|---|---|---|
| 50% | 1564546 | 5 | 4.96 | 99.91% | 99.56% |
| 100% | 3323709 | 10 | 9.98 | 99.18% | |
| 150% | 5212616 | 15 | 15.02 | 99.60% | |

The accuracy study was performed for % recovery of tipranavir. The % recovery was found to be 99.56% (NLT 98% and NMT 102%)

4 Precision:

a)Repeatability

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.

b)Intermediate precision/Ruggedness

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.

5.Repeatability :

The precision study was performed for five injections of tipranavir. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD.

**Table.No.4.Showing% RSD results for tipranavir**

| | Peak name | RT | Area | Height | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | tipranavir | 1.989 | 3142617 | 358347 | 1516 | 1.2 |
| 2 | tipranavir | 1.989 | 3174044 | 361853 | 1489 | 1.2 |
| 3 | tipranavir | 1.988 | 3179773 | 361287 | 1483 | 1.2 |
| 4 | tipranavir | 1.988 | 3153022 | 358451 | 1490 | 1.2 |
| 5 | tipranavir | 1.988 | 3145507 | 361788 | 1476 | 1.2 |
| 6 | tipranavir | 1.987 | 3144053 | 361472 | 1496 | 1.2 |
| mean | | 2.0 | 3161900 | 361440 | | |
| Std.dev | | 0.0 | 15288.3 | | | |
| %RSD | | 0.1 | 0.5 | | | |

The Method precision study was performed for the %RSD of tipranavir was found to be 0.5 (NMT 2).

6 Intermediate precision/Ruggedness:

The intermediate precision study was performed for five injections of tipranavir. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

**Table.No.5. Showing results for intermediate precision of tipranavir**

| | Peak name | RT | Area | Height | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | tipranavir | 1.988 | 3142617 | 358347 | 1513 | 1.2 |
| 2 | tipranavir | 1.987 | 3174044 | 361853 | 1485 | 1.2 |
| 3 | tipranavir | 1.987 | 3179773 | 361287 | 1496 | 1.2 |
| 4 | tipranavir | 1.987 | 3153022 | 358451 | 1483 | 1.3 |
| 5 | tipranavir | 1.986 | 3145507 | 361788 | 1497 | 1.3 |
| 6 | tipranavir | 1.985 | 3144053 | 361472 | 1495 | 1.2 |
| mean | | 2.0 | 3161900 | 361440 | | |
| Std.dev | | 0.0 | 15288.3 | | | |
| %RSD | | 0.1 | 0.5 | | | |

The intermediate precision was performed for %RSD of tipranavir was found to be 0.6 (NMT 2).

**7. Detection limit (LOD)**

**Table .No.6 Showing results for Limit of Detection**

| | Peak name | RT | Area | Height | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | Tipranavir LOD | 1.973 | 726628 | 73311 | 1459 | 1.12 |

The LOD performed for tipranavir was found to be 1.97.

**8.Quantitation limit**

(LOQ's)

**Table .No.7.Showing results for Limit of quantification**

| | Peak name | RT | Area | Height | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | Tipranavir LOQ | 1.977 | 1466599 | 149050 | 1405 | 1.13 |

The LOQ was performed for tipranavir found to be 1.977.

9. Robustness:

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for tipranavir The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

| | Peak name | RT | Area | Height | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | Tipranavir LOQ | 1.747 | 3105967 | 356959 | 2318 | 1.14 |

**Table no.8.showing less flow rate 0.8ml/min**

**Table no.9.less flow rate 1.2 ml/min**

| | Peak name | RT | Area | Height | USP plate count | USP tailing |
|---|---|---|---|---|---|---|
| 1 | Tipranavir LOQ | 1.985 | 3581323 | 366237 | 2375 | 1.14 |

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

**Table.No10. Showing system suitability results for tipranavir**

| S. No | Flow rate (ml/min) | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.8 | 2318 | 1.4 |
| **2** | **1** | **2568** | **1.3** |
| 3 | 1.2 | 2375 | 1.4 |

**Table.No.11. Showing system suitability results for tipranavir**

| S. No | Change in organic composition in the mobile phase | System suitability results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 5 % less | 2318 | 1.4 |
| 2 | ***Actual** | **2568** | **1.3** |
| 3 | 5 % more | 2319 | 1.4 |

## Conclusion:

Hence the suggested RP-HPLC method can be used for routine analysis of Tipranavir in API and Pharmaceutical dosage form.

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