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Research Article

A Novel RP-HPLC Method for the Quantification of Tipranavir In Formulations

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Tipranavir in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Water: Tri ethyl Amine (TEA) 50:25:25% (V/V). The UV detection wavelength was 277nm and 20µl sample was injected. The retention time for Tipranavir was 3.32min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Tipranavir in tablet dosage form and bulk drug.

Keywords: Tipranavir, RP-HPLC, UV detection, recovery, precise, 277 nm.

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 two 250 mg capsules together with 200 mg of ritonavir twice daily.

Tipranavir has the ability to inhibit the replication of viruses that are resistant to other protease inhibitors and it is recommended for patients who are resistant to other treatments. Resistance to tipranavir itself seems to require multiple mutations.

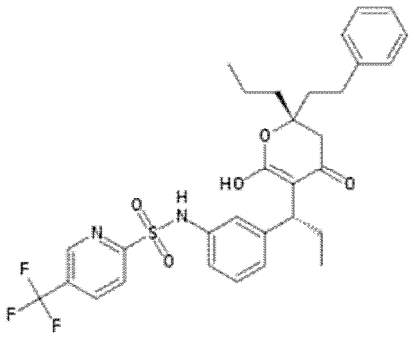

Fig. 1: Structure of Tipranavir

Tipranavir was administered orally in conjunction with low-dose ritonavir (ritonavir-boosted tipranavir).Ritonavir-boosted tipranavir was used in patients who are highly treatment-experienced or infected with HIV-1 resistant to multiple PIs. It should not be used without low-dose ritonavir. Oral solution can be used.Capsules can be alternatively given to children who can reliably swallow a capsule.. Medication errors can be avoided, by taking extra care in calculating the dose, transcribing the medication order, and dispensing the prescription.

EXPERIMENTAL

Materials

Working standard of Tipranavir was obtained from well reputed research laboratories. HPLC grade water, Methanol, TEA was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer

was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Tipranavir were scanned in the range of 200 -400 nm against mobile phase as a blank. Tipranavir showed maximum absorbance at 277 nm. So the wavelength selected for the determination of Tipranavir was 277 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil100-5 C18. 250×4.6mm, manual injector Rheodyne valves with 20µL fixed loop, PEAK LC softwarewas used.

The mobile phase consisted of Methanol: Water: TEA:50:25:25(v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 277 nm with 10min runtime.

Standard and sample solutions

A 10 mg amount of Tipranavir reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations of 100ppm were prepared by serial dilution of this solution . 4 ml was taken from this solution and made upto 10 ml using mobile phase in a volumetric flask.

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Tipranavir was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 40 ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In allcases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Table 1: System suitability parameters of Tipranavir

Mobile phase	Methanol: Water: TEA: 50:25:25 (v/v)
Pump mode	Isocratic
pH	5.95
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	277nm
Injection Volume	20 µl
Flow rate	1 ml/min
Run time	10 minutes
Retention Time	3.32 minutes

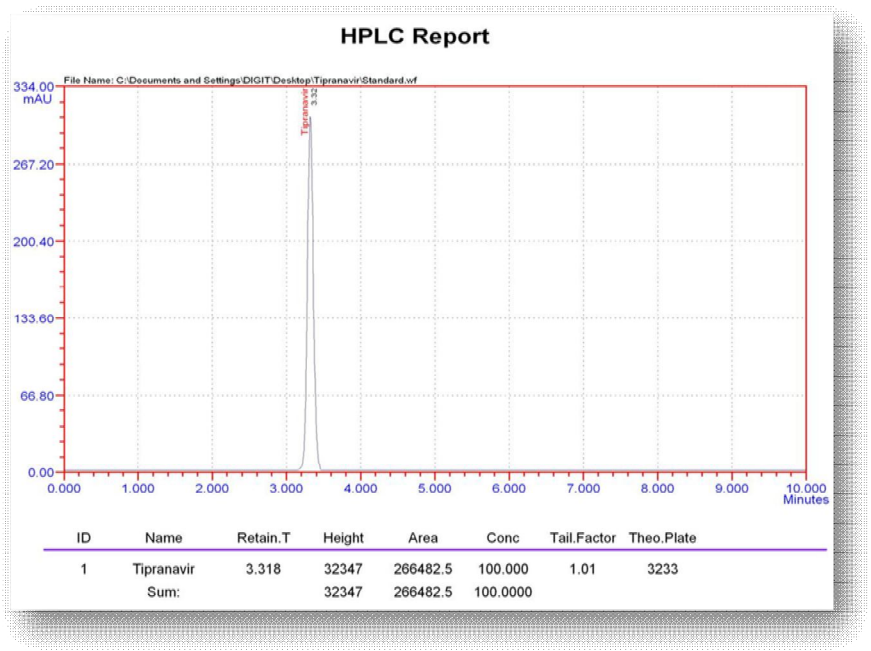

Fig. 2: Standard chromatogram of Tipranavir

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 10, 20, 30, 40, 50, 60, 70 and 80 ppm for Tipranavir. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 1638 + 6739x (r= 0.999). Linearity values can shown in Table: 2.

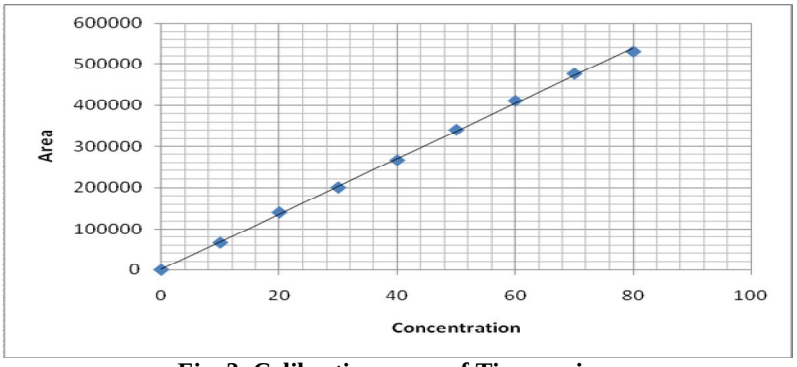

Fig. 3: Calibration curve of Tipranavir

Table 2: Linearity results of Tipranavir

Level	Concentration of Tipranavir in ppm	Peak Area
Level 1	10	66931
Level 2	20	140149
Level 3	30	200237
Level 4	40	266482
Level 5	50	340963
Level 6	60	410691
Level 7	70	475382
Level 8	80	529854
Range 5 ppm to 30 ppm	SLOPE INTERCEPT CORREALATION COEFFICIENT	6739 1638 0.999

Precision

To study precision, six replicate standard solutions of Tipranavir (40 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Table 3: Intraday Precision Results for Tipranavir

Sample	Conc. (in ppm)	Injection No.	Peak Areas	RSD (Acceptance criteria ≤ 2.0%)
Tipranavir	40	1	266482	0.798
		2	261861	
		3	265736	
		4	266495	
		5	267106	
		6	267908	

Table 4: Inter day Precision results of Tipranavir

Sample	Conc. (in ppm)	Injection No.	Peak Areas	RSD (Acceptance criteria ≤ 2.0%)
Tipranavir	40	1	265930	1.03
		2	269321	
		3	267638	
		4	264195	
		5	266030	
		6	261396	

Limit of Detection and Limit of Quantification

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.02 ppm dilution Peak was not clearly observed, based on which 0.02ppm is considered as Limit of Detection and Limit of Quantification is 0.066 ppm.

Table 5: LOD and LOQ results of Tipranavir

Parameter	Measured Value
Limit of Quantification	0.066 ppm
Limit of Detection	0.02ppm

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Recovery

Recovery test was performed at 3 different concentrations i.e. 20ppm, 40ppm, 60ppm. Results are given in table.7.

Table 6: Robustness results of Tipranavir

S.No	Parameter	Condition	Area	% of Change
1	Standard	Standard conditions	266482	……..
2	Mobile phase	Methanol : WATER: TEA : 55:20:25	262387	1.53
3	Mobile phase pH	5.7	267394	0.34
4	Wavelength	272 nm	266852	0.14

Table 7: Recovery results of Tipranavir

Recovery	Conc of sample (ppm)	Recovery(ppm)	% of recovery
50%	20	19.82	99.1
100%	40	39.86	99.65
150%	60	60.46	100.77

Table 8: Formulation Analysis

S.NO	Tablet	Dosage	Sample conc	Sample estimated	% of Drug Estimated in Tablet
1	Aptivus	250mg	40ppm	39.73	99.325

CONCLUSION

The proposed method for the assay of Tipranavir in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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