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Method development & validation of a drug ritonavir by RP-HPLC method

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ABSTRACT

A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Ritonavir in bulk and pharmaceutical dosage form dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cm x 4.6mm i.d. column with UV detection at 210 nm and Acetonitrile: Methanol with 68:32 ratio at a flow rate of 1.0 ml/ min. The proposed method was successfully applied to the determination of Ritonavir in bulk and pharmaceutical dosage form. The method was linear over the range of 6-14µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.09 µg/ml and quantification was found to be 0.27 µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.

Keywords: RP-HPLC, Ritonavir, Method development and validation, ICH Guidelines.

INTRODUCTION

Ritonavir is an HIV protease inhibitor that interferes with the reproductive cycle of HIV.[1] Although it was initially developed as an independent antiviral agent, it has been shown to possess advantageous properties in combination regimens with low-dose ritonavir and other protease inhibitors.[2-7] It is now more commonly used as a booster of other protease inhibitors and is available in both liquid formulation and as capsules.[8-11] While ritonavir is not an active antiviral agent against hepatitis C virus (HCV) infection, it is added in combination therapies indicated for treatment of HCV infections as a booster.[12-17] Ritonavir is a potent CYP3A inhibitor that increases peak and trough plasma drug concentrations of other protease inhibitors

such as Paritaprevir and overall drug exposure.[18-20] American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) guidelines recommend ritonavir-boosted combination therapies as a first-line therapy for HCV Genotype 1a/b and 4 treatment-naïve patients with or without cirrhosis [21-23].

The IUPAC Name of Ritonavir is 1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate[4]

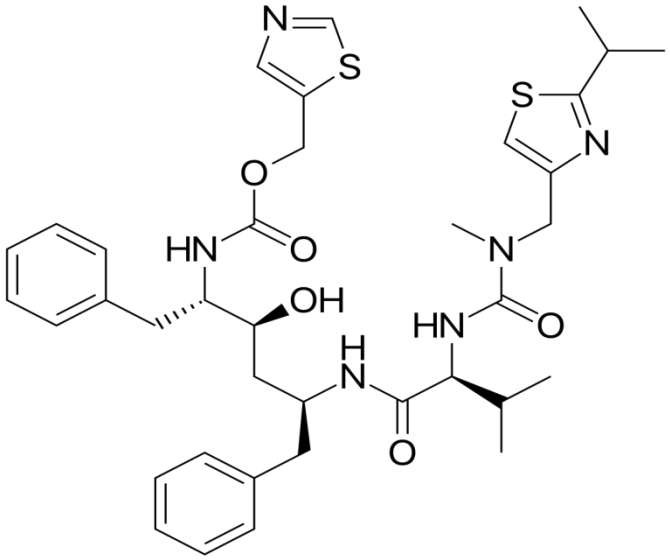


Fig 1: Chemical Structure of Ritonavir[25]

MATERIALS AND METHODS

HPLC Instrumentation & Conditions

The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis

25 mg of Ritonavir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Ritonavir, so that the same wave number can be utilized in HPLC UV detector for estimating the Ritonavir. While scanning the Ritonavir solution we observed the maxima at 210nm. The UV spectrum has been recorded on T60-LABINDIA make UV – Vis spectrophotometer model UV-2450.

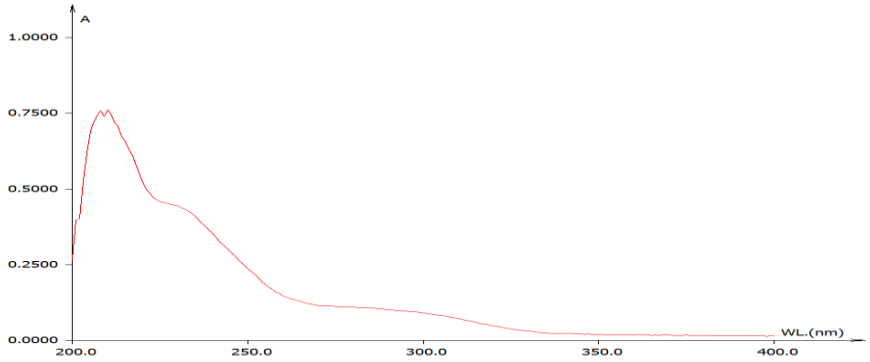


Fig 2: UV Spectrum

Optimized Chromatographic Conditions

- **Column:** Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d..
- **Mobile Phase:** Acetonitrile: Methanol (68:32 v/v).
- **Flow Rate :** 1.0ml/minute
- **Wave length :** 210 nm
- **Injection volume :** 20µl
- **Run time:** 06 mins.
- **Column temperature :** Ambient
- **Sampler cooler :** Ambient

MOBILE PHASE PREPARATION

Mobile phase was prepared by taking Acetonitrile: Methanol (68:32 v/v). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

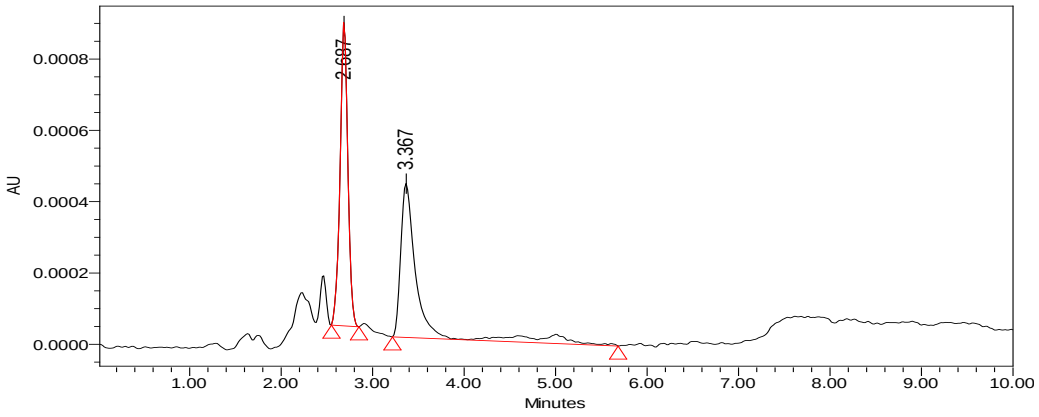
SAMPLE & STANDARD PREPARATION FOR THE ANALYSIS

25 mg of Ritonavir standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

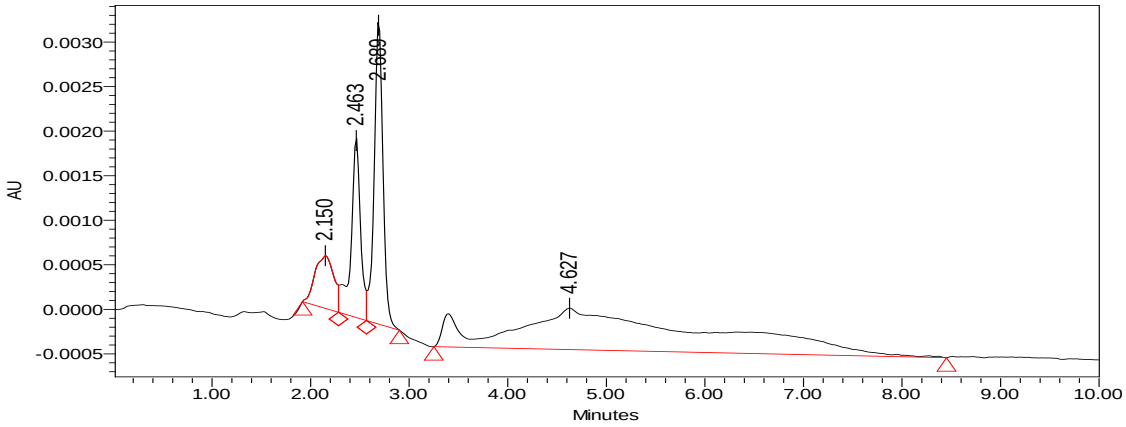
RESULT AND DISCUSSION

Table-1:Trials for method development

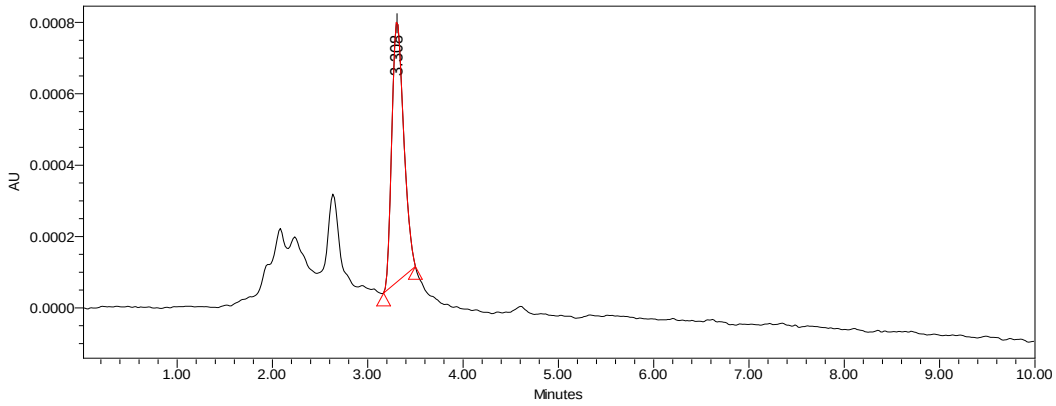
Column Used	Mobile Phase	Flow Rate	Run Time	Wave length	Observation	Result
Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.	ACN : Water = 40 : 60	1.0 ml/min	10 mins	210 nm	Peak broken	Method rejected
Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.	ACN : Water = 30 : 70	0.8 ml/min	10 mins	210 nm	Very low response	Method rejected
Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.	ACN: water = 35 : 65	1.0 ml/min	10 mins	210 nm	Tailing peak	Method rejected
Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.	ACN: phosphate buffer = 40:60	1.0 ml/ min	6 mins	210 nm	Broad Peak	Method rejected
Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d.	Acetonitrile: Methanol = 68:32	1.0 ml/min	6 mins	210 nm	Nice peak	Method accepted



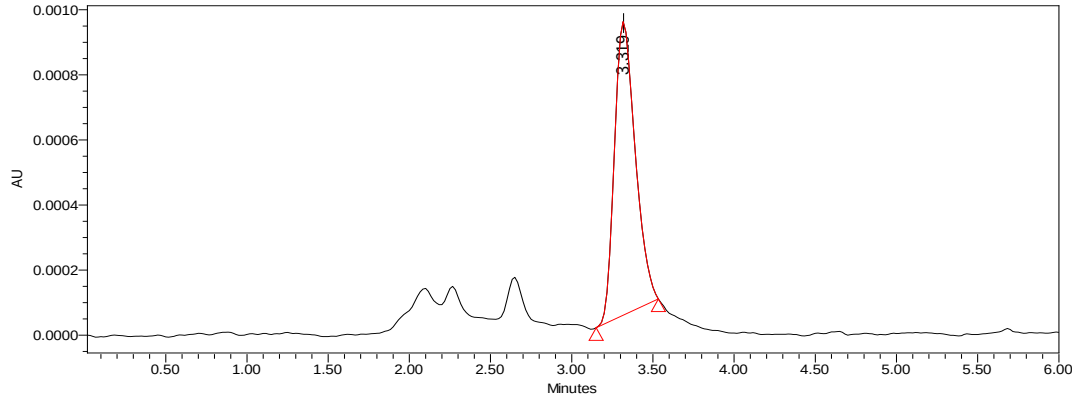
Trial-1



Trial-2



Trial-3



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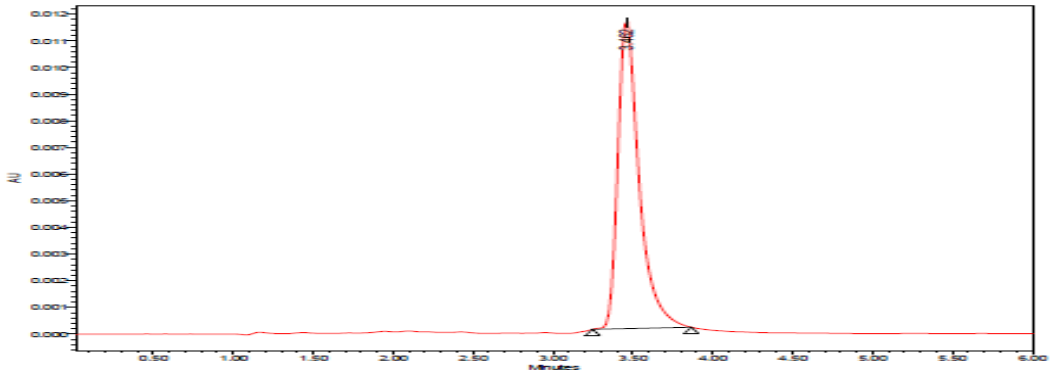

Trial-5

Table 2: Peak results

RT	Area	Theoretical Plates	Tailing factor
3.462	289561	2458	1.27

METHOD VALIDATION

Accuracy: ***Recovery study:*** To decide the exactness of the proposed strategy, recuperation thinks about were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of Ritonavir were taken and added to the pre-dissected detailing of fixation 10µg/ml. From that rate recuperation esteems were ascertained. The outcomes were appeared in Table-3.

Table-3: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S1 : 80 %	8	8.157	595625	101.962	Mean= 101.387%
S2 : 80 %	8	8.099	591457	101.237	S.D. = 0.516599
S3 : 80 %	8	8.077	589875	100.962	% R.S.D.= 0.509532
S4 : 100 %	10	10.077	734587	100.77	Mean= 100.43%
S5 : 100 %	10	9.948	725268	99.48	S.D. = 0.833727
S6 : 100 %	10	10.104	736524	101.04	% R.S.D.= 0.830157
S7 : 120 %	12	11.989	872949	99.908	Mean= 100.6997%
S8 : 120 %	12	12.190	887456	101.583	S.D. = 0.841254
S9 : 120 %	12	12.073	878975	100.608	% R.S.D.= 0.835409

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Ritonavir (API) the percent relative standard deviations were calculated for Ritonavir is presented in the Table-4.

Table-4: Repeatability Results of Precision

HPLC Injection Replicates of Ritonavir	Retention Time	Peak Area
Replicate – 1	3.461	726541
Replicate – 2	3.461	724857
Replicate – 3	3.462	723541
Replicate – 4	3.461	725268
Replicate – 5	3.459	728984
Replicate -6	3.459	725745
Average	3.4605	725822.7
Standard Deviation	0.001224745	1841.86
% RSD	0.0353921	0.253762

Intra day & Inter day: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Ritonavir revealed that the proposed method is precise.

Table-5: Results of Intra day & Inter day

Conc. Of Ritonavir (API) (µg/ml)	Observed Conc. Of Ritonavir (µg/ml) by the proposed method			
	Intra day		Inter day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.02	1.05	7.96	1.06
10	10.10	0.96	10.06	0.99
12	11.89	0.85	12.03	0.92

Linearity and Range

Linearity range was found to be 6-14µg/ml for Ritonavir. The correlation coefficient was found to be 0.999, the slope was found to be 72353 and intercept was found to be 5437 for Ritonavir [21].

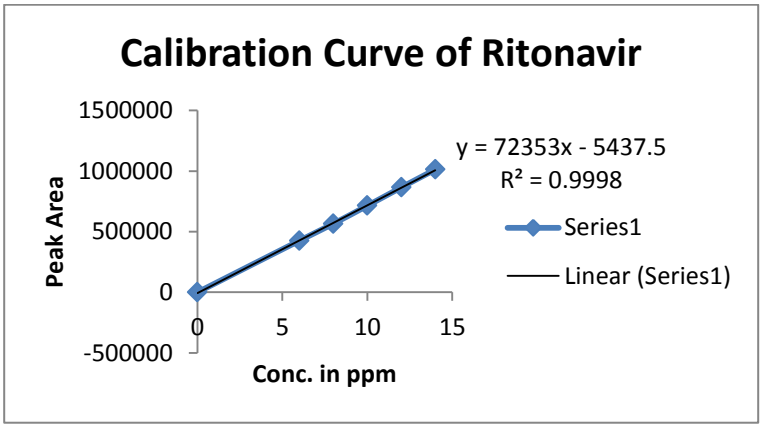

Fig-3: Calibration curve of Ritonavir (API)

Table-6: Linearity Results of Ritonavir

CONC.	PEAK AREA
0	0
6	425874
8	565872
10	714542
12	865632
14	1013121

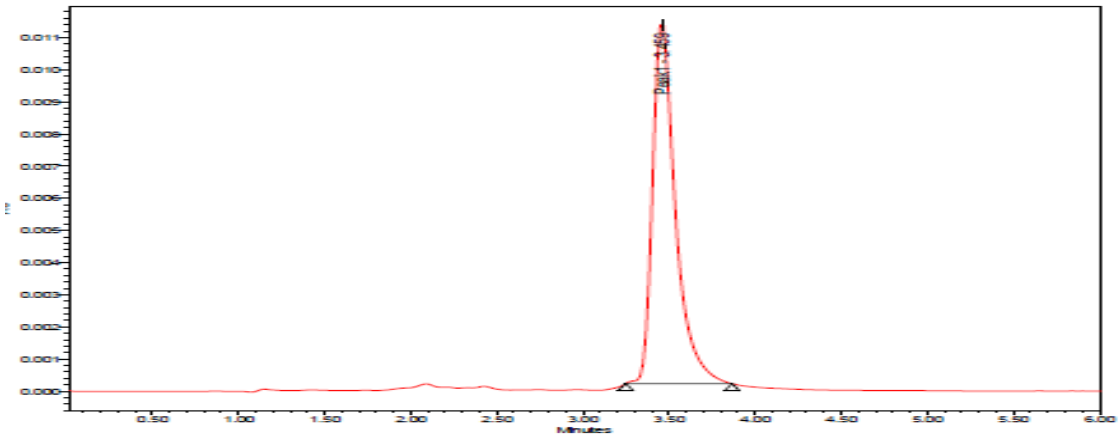

Fig 4: Calibration of Ritonavir concentration in 6 ppm

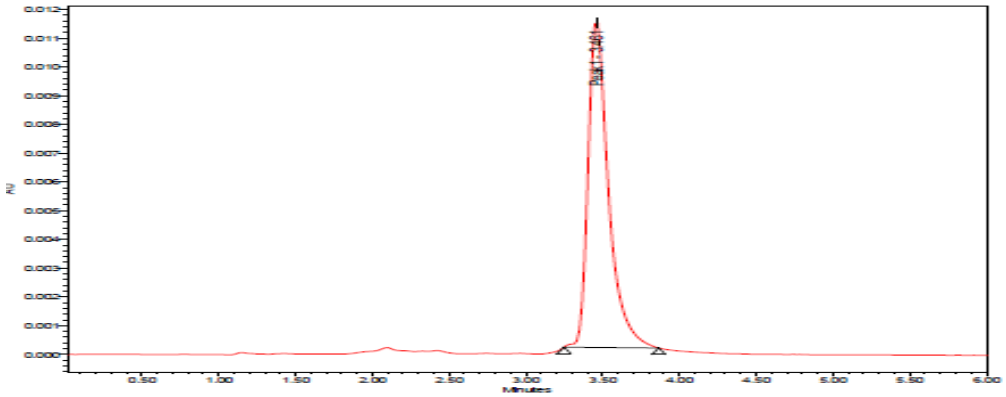

Fig 5: Calibration of Ritonavir concentration in 8 ppm

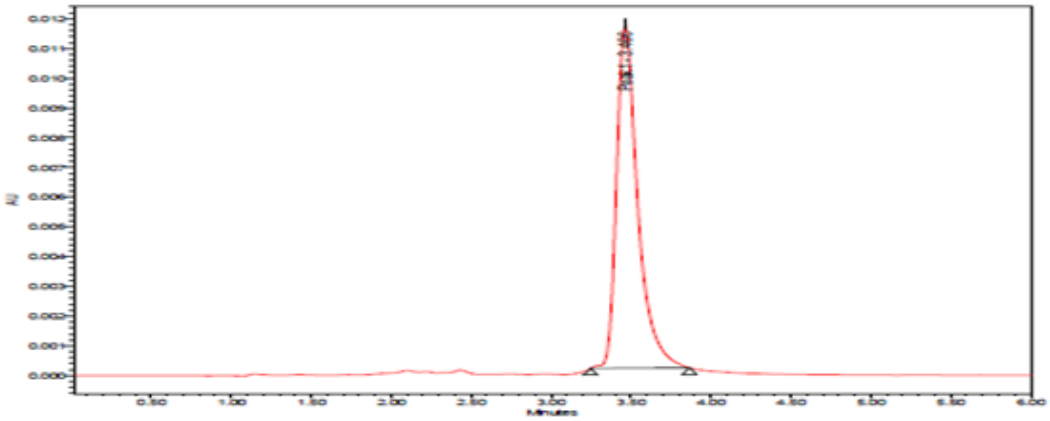

Fig 6: Calibration of Ritonavir concentration in 10 ppm

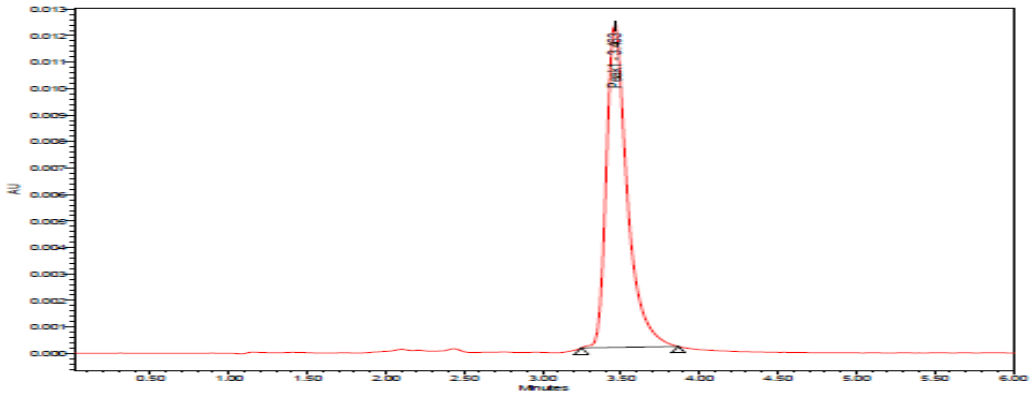

Fig 7: Calibration of Ritonavir concentration in 12 ppm

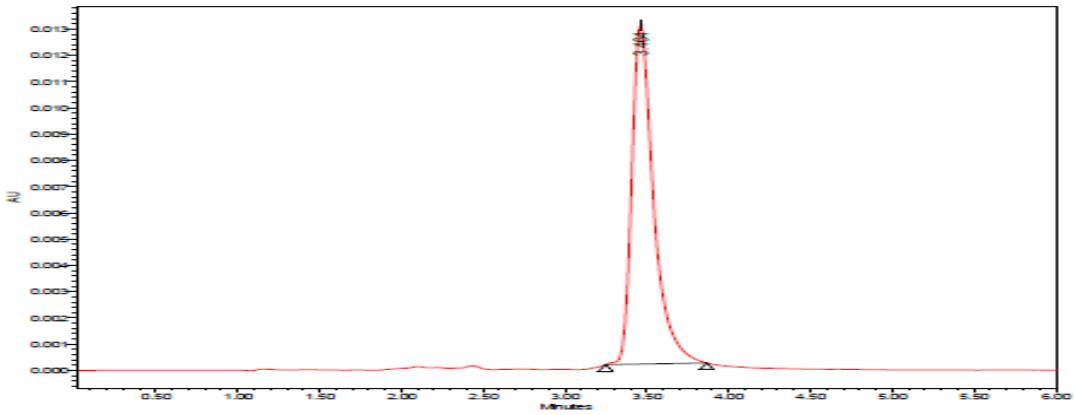

Fig 8: Calibration of Ritonavir concentration in 14 ppm

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.27 µg/ml respectively.

CONCLUSION

A delicate and specific, sensitive RP-HPLC strategy has been created and approved for the investigation of Ritonavir API.

Facilitate the proposed RP-HPLC strategy has amazing affectability, exactness and reproducibility.

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