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Original Article

Determination of Oxazolidinone Impurity in Ritonavir and Lopinavir Tablet Formulation by Using RP-LC Technique

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Abstract *- A highly sensitive method for the determination of oxazolidinone impurity in Ritonavir tablet formulations using RP-HPLC has been presented in the present paper. Quantification of oxazolidinone impurity content in Ritonavir tablet formulation samples by HPLC with UV Detector. Oxazolidinone was determined by the RP-HPLC method using YMC Pack ODS-AQ (150 x 4.6mm, 3µm) column as stationary phase. The mobile phase consisted of phosphate buffer, acetonitrile, methanol and tetrahydrofuran in the ratio of 625:175:100:100 v/v/v/v is used as Mobile phase-A and phosphate buffer, acetonitrile, methanol and tetrahydrofuran in the ratio of 400:200:200:200 v/v/v/v used as Mobile Phase-B, with the help of the gradient elution. Column temperature maintained 40°C and sample cooler temperature 25°C, Injection volume 50µL, the Flow rate was 1.5 ml/min and the separated oxazolidinone was detected using UV detector at the wavelength of 240 nm and run time was 45mintues. The method validation has been carried out as per International Conference on Harmonization guidelines (ICH). Limit of quantitation (LOQ) was found 0.24ppm for oxazolidinone.*

Keywords *- Oxazolidinone impurity, Ritonavir tablet formulation, RP-LC method, validation and limit of quantitation.*

I. INTRODUCTION

Synthesis of drug substances often involves the use of reactive reagents, and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity and are to be controlled based on the maximum daily dose [1]. These limits generally fall at low µg/mL levels. HPLC, GC methods (or final drug substance methods) are suitable for their determination. Their applications are oriented towards the potential identification and quantitation of trace levels of impurities in drug substances [2].

Ritonavir (RTV) is a selective, competitive and reversible inhibitor of both HIV-1 and HIV-2 proteases. It is widely used in the treatment of AIDS and particularly to inhibit the liver enzyme, viz., cytochrome P450-3A4 (CYP3A) [3]. It is not only used on its own as an antiretroviral but also a booster of other anti-HIV drugs to slow and/or prevent the onset of AIDS. Chemically, RTV is (5S,8S,10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis (phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid,5-thiazolylmethyl ester.

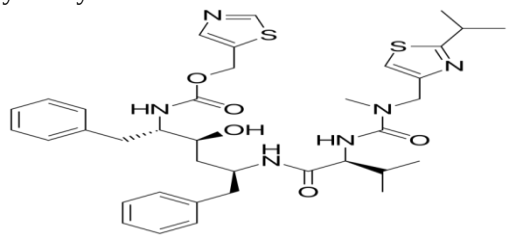

Fig. 1 Chemical structure of ritonavir

A. Impurity structure

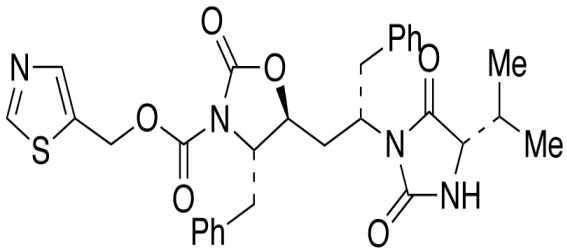

Fig. 2 Chemical structure of oxazolidinone impurity

The literature survey reveals that various analytical techniques like HPLC methods were published for the determination of antiretrovirals, including ritonavir in different formulations [4–7]. HPTLC was used for

simultaneous determination of ritonavir and lopinavir in capsules [8]. A few CE and LC-MS methods were reported for analysis of ritonavir and its metabolites in biological fluids [9–35]. International Pharmacopoeia (Ph.Int.) describes an LC method to separate ritonavir and its impurities. However, no brand names of columns (L1) were mentioned in pharmacopoeial texts, due to which analysts often face difficulty in selecting a suitable stationary phase.

In literature, no analytical method was reported for the determination of oxazolidinone impurity in Ritonavir tablet formulation samples. Hence the author was aimed towards the development of rapid, specific and robust methods for the determination of oxazolidinone impurity in Ritonavir tablet formulation at trace level concentration.

II. EXPERIMENTAL

A. Chemicals and Reagents

Oxazolidinone was purchased from Sigma-Aldrich., Mumbai, India. Potassium dihydrogen orthophosphate, Acetonitrile, Methanol, Tetrahydrofuran HPLC grade water were procured from Merck, India.

B. Preparation of Buffer

Weighed accurately 4.1g of potassium dihydrogen orthophosphate transferred into a 1000mL beaker dissolved and diluted to volume with 1000mL milli-Q water. Filter through 0.45µ membrane filter paper.

C. Mobile Phase-A

Mixed phosphate buffer, acetonitrile, methanol and tetrahydrofuran in the ratio of 625:175:100:100 v/v/v/v accurately. Filter through 0.45µ membrane filter paper.

D. Mobile Phase-B

Mixed phosphate buffer, acetonitrile, methanol and tetrahydrofuran in the ratio of 400:200:200:200 v/v/v/v accurately. Filter through 0.45µ membrane filter paper.

E. Preparation of Diluent

Mixed phosphate buffer and acetonitrile in the ratio of 500:500 (%v/v) accurately.

F. Preparation of Oxazolidinone Stock Solution

Accurately weighed and transferred 1.0 mg of oxazolidinone standard into a 100mL volumetric flask. Added 50ml of diluent and sonicated for 5 minutes. Made up the volume with diluent and mixed well.

G. Preparation of Standard Stock Solution

Accurately weighed and transferred 50 mg of ritonavir standard into a 50mL volumetric flask. Added 25ml of diluent and sonicated for 5 minutes. Made up the volume with diluent and mixed well.

H. Preparation of Standard Solution

Transferred 5mL of ritonavir standard stock solution into a 50mL volumetric flask and made up to the volume with diluent and mixed well.

I. Preparation of System Suitability Solution

Transferred 5mL of ritonavir standard stock solution and 1.5 mL of oxazolidinone standard stock solution into a 10mL volumetric flask and made up to the volume with diluent and mixed well.

J. Preparation of Sample Solution

Weighed and transferred 10 tablets into a 250ml volumetric flask, added 100ml diluent and sonicated for 20minutes; after cooling, made up to the volume with diluent. Centrifuge the solution at 4000 rpm for 10minutes. Further diluted 5ml of this solution to 10ml with diluent and mixed well.

K. Preparation of placebo solution

Transferred placebo powder equivalent to 250 mg of ritonavir into a 250ml volumetric flask, added 100ml diluent and sonicated for 20minutes after cooling made up to the volume with diluent. Centrifuge the solution at 4000 rpm for 10minutes. Further diluted 5ml of this solution to 10ml with diluent and mixed well.

L. Chromatographic conditions

RP-HPLC analysis was carried out on Shimadzu LC-2050 series, Lab solution software. YMC Pack ODS-AQ (150x4.6mm, 3µm) column as stationary phase. The mobile phase consisted of phosphate buffer, acetonitrile, methanol and tetrahydrofuran in the ratio of 625:175:100:100 v/v/v/v is used as Mobile phase-A and phosphate buffer, acetonitrile, methanol and tetrahydrofuran in the ratio of 400:200:200:200 v/v/v/v used as Mobile Phase-B, with the help of the gradient elution. Column temperature maintained 40°C, and sample cooler temperature 25°C, Injection volume 50µL, the Flow rate was 1.5 ml/min, and the separated oxazolidinone was detected using UV detector at the wavelength of 240 nm and run time was 45mintues respectively.

III. METHOD DEVELOPMENT

A. Method Optimization Parameters

An understanding of the nature of drug substance and drug product (functionality, acidity, or basicity), the synthetic process, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC.

In addition, successful method development should result in a robust, simple and time-efficient method that is capable of being utilized in the manufacturing setting.

B. Selection of Wavelength

The sensitivity of the HPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives a good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 240 nm from the absorption spectrum.

C. Selection of stationary phase

Proper selection of the stationary phase depends on the nature of the sample and chemical profile. The drug selected for the present study was a polar compound and could be separated either by normal phase chromatography or reverse-phase chromatography. From the literature survey, it was found that different C18 columns could be appropriately used for the separation of related substances for Ritonavir and Lopinavir tablet formulation.

D. Selection of mobile phase

Different mobile phase and stationary phases were employed to develop a suitable LC method for the quantitative determination of oxazolidinone impurity in Ritonavir and Lopinavir tablet formulation. A number of column chemistries supplied by different manufacturers and different mobile phase compositions were tried to get good peak shapes and selectivity for the impurities present in Ritonavir and Lopinavir tablet formulation.

E. Method Validation

a) Specificity

1) Blank interference

Blank was prepared and injected as per the test method. It was observed that no blank peaks were interfering with analytical peaks.

2) Placebo interference

Placebo solutions were prepared in duplicate and injected as per the test method. It was observed that no placebo peaks were interfering with analytical peaks.

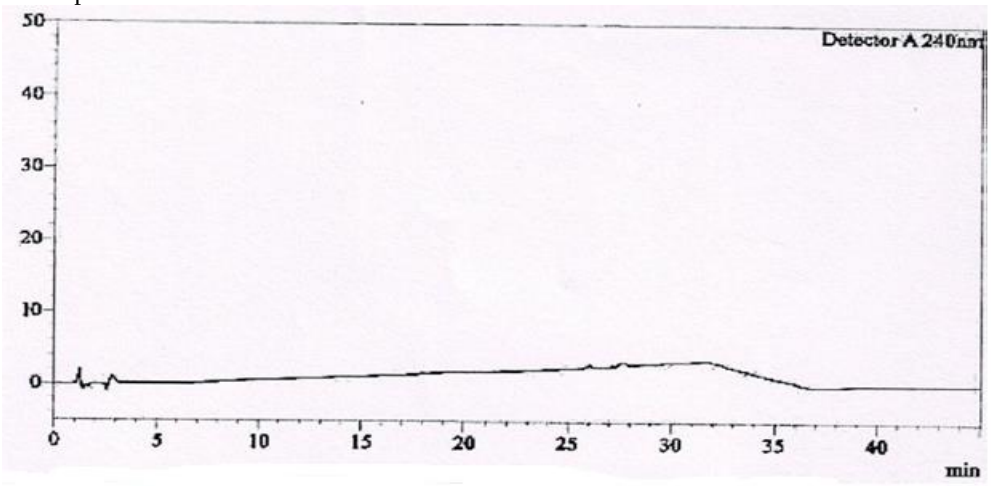

Fig. 3 Typical chromatogram of blank

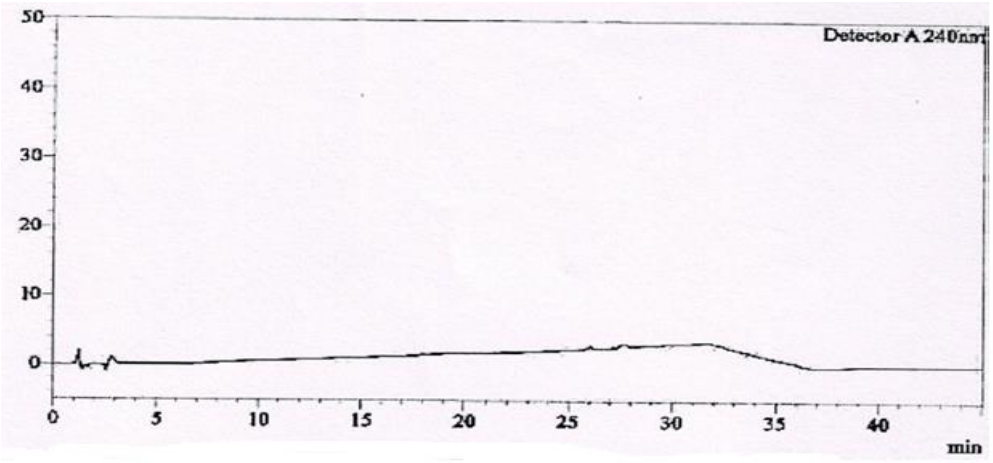

Fig. 4 Typical chromatogram of placebo

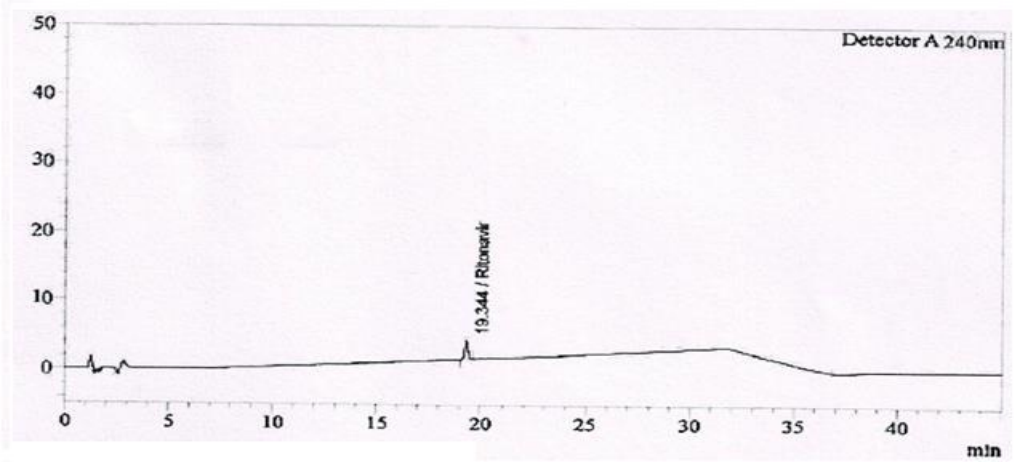

Fig. 5 Typical chromatogram of standard

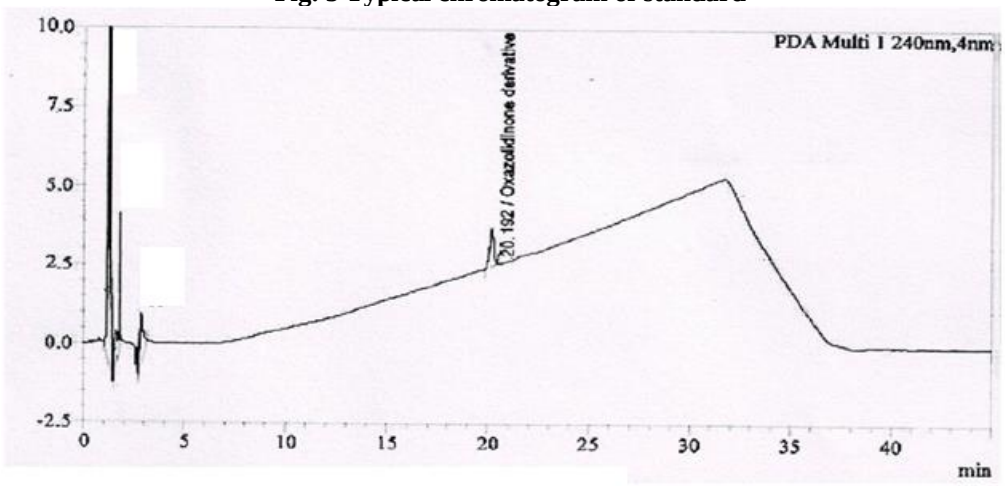

Fig. 6 Typical chromatogram of oxazolidinone impurity

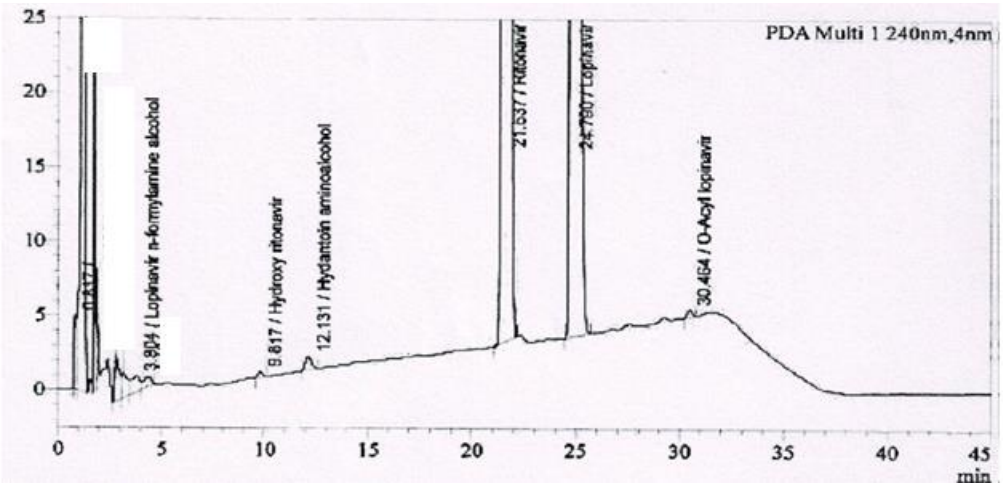

Fig. 7 Typical chromatogram of as such sample

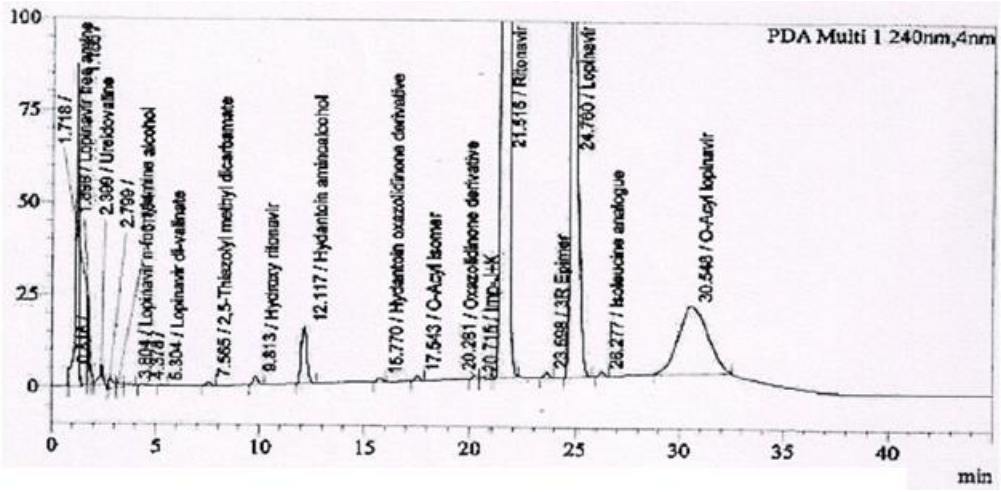

Fig. 8 Typical chromatogram of the spiked sample

It was observed that no interference of blank and placebo at oxazolidinone impurity retention time.

F. Precision

a) System Precision

Perform the analysis of reference solution (Diluted standard) six times and determine the percentage relative standard deviation of peak area of replicate injections of ritonavir.

Table 1. System precision data for ritonavir

Inj. No	Ritonavir
1	35914
2	35732
3	35624
4	35928
5	35737
6	35929
Mean (%)	**35811**
% RSD	**0.40**

The %RSD of peak area for ritonavir was found to be 0.4% which is below 5.0%, indicating that the system gives precise results.

b) Method Precision

Precision was determined by injecting six sample solutions spiked oxazolidinone impurity at the specification level. The samples were prepared as per the method, and the result for the precision study is tabulated in Table: 2.0.

Table 2. Results of method precision

Preparations	Oxazolidinone
1	0.24
2	0.24
3	0.24
4	0.25
5	0.25
6	0.24
Mean (%)	0.24
% RSD	**2.1**

The method precession was performed with six replicate solutions of standard solutions prepared and the system suitability parameters found were within the acceptance criteria

G. Limit of quantitation (LOQ) & Limit of detection (LOD)

a) Limit of detection

A solution containing 0.0801 µg/ml of oxazolidinone impurity was injected. The worst found signal to noise ratio for each peak was greater than 3 in each injection.

b) Limit of Quantitation

A solution containing 0.2426 µg/ml of oxazolidinone impurity was injected. The worst found signal to noise ratio for each peak was greater than 10 in each injection.

Table 3. LOD & LOQ concentrations and S/N values for oxazolidinone impurity

Name of the impurity	Concentration in ppm		Signal to noise ratio value	
	LOD	LOQ	LOD	LOQ
Oxazolidinone	*0.0801*	*0.2426*	*3.1*	*16.7*

H. Linearity and Range

The linearity is determined by injecting the solutions in duplicate oxazolidinone ranging from LOQ to 250% of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Report the linearity range as the range for determining the oxazolidinone impurity.

Table 4. Linearity of detector response oxazolidinone

Level	Concentration (ppm)	Mean area
LOQ	0.2426	2811
50%	0.7581	9634
80%	1.2129	15860
100%	1.5162	19753
120%	1.8194	23870
150%	2.2742	29621
200%	3.0323	40014
250%	3.7904	50209
Correlation coefficient		0.9999
% Y-intercept		-2.28
Slope		13340.2163
Intercept		-451.2678

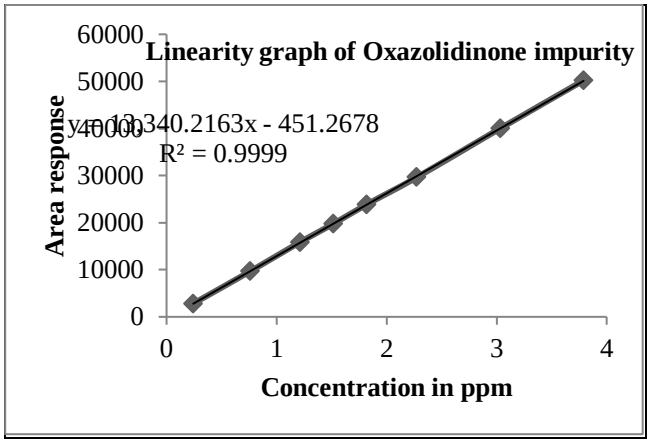

Fig. 9 linearity of detector response for oxazolidinone

I. Accuracy

Recovery of oxazolidinone impurity was performed. The sample was taken, and varying amounts of oxazolidinone impurity representing LOQ to 150 % of specification level were added to the flasks. The spiked samples were prepared as per the method, and the results are tabulated in Table 5.0.

Table 5. Accuracy study of oxazolidinone

S.No.	Theoretical (%)	% Mean Recovery
		Oxazolidinone
1	LOQ	81.5
2	50	90.0
3	100	96.6
4	120	98.0
5	150	98.1

IV. RESULTS & DISCUSSION

The specificity of the developed LC method was indicated by oxazolidinone impurity (1.5µg/mL) with respect to 0.5mg/mL of ritonavir was injected separately, and S/N ratios were recorded. These solutions were further diluted to achieve the signal-to-noise (S/N) ratios at about 3 and 10 for determining LOD and LOQ, respectively, for both methods. The precision of the methods was checked by injecting LOQ solutions six times. The value of RSDs for the area of oxazolidinone impurity was calculated.

The intermediate precision of the method was also verified on six different days in the same laboratory using the LOQ level solutions. The low RSD values ensured the precision of the developed method. Linearity test solution for oxazolidinone impurity was prepared individually at six concentration levels in the range of LOQ to 250% of the specification level. LOQ and sixth levels were injected six times, and the other four levels were injected thrice. The average peak areas versus concentrations were subjected to least-squares linear regression analysis.

The derived correlation coefficients were above 0.9999, indicating the best fitness of the linearity curves of the developed method. Standard addition experiments were conducted in triplicate preparations to determine the accuracy of the methods at LOQ to 150% level were determined. The recoveries were found to be in the accepted range.

The system suitability of the method was ensured by getting the resolution 2.5 between oxazolidinone impurity and Ritonavir. Ritonavir and Lopinavir tablet formulation at trace level concentration have been developed and validated as per ICH guidelines [36].

V. CONCLUSION

The proposed RP-LC method that can quantify oxazolidinone impurity in Ritonavir and Lopinavir tablet formulation at trace level concentration have been developed and validated as per ICH guidelines. The effectiveness of the method was ensured by the specificity, precision, LOD, LOQ, linearity and accuracy. Hence, the method is well suited for their intended purposes and can be successfully applied for the release testing of Ritonavir and Lopinavir tablet formulation into the market.

VI. ACKNOWLEDGEMENT

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VII. CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest

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