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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RITONAVIR BY RP-HPLC METHOD

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ABSTRACT

Background: Ritonavir is an antiviral drug and is principally used as a booster for increasing the bioavailability of alternative protease inhibitors like Atazanavir salt and Lopinavir. **Aims:** Quality assessment of the new dose sort of protease inhibitor i.e. tablets, is extremely essential, thus sensitive, straightforward and precise strategies were measure developed for quantification of protease inhibitor in bulk and tablet dosage forms. **Materials and Methods**: The strategies square measure valid in step with international conference of harmonization (ICH) tips. **Result:** A simple, precise and reproducible Reverse Phase High Performance Liquid Chromatography method was developed and validated for estimation of Ritonavir in tablet dosage form. The chromatographic separation was done by cosmosil C18 (250 mm x 4.6 ID, Particle size-5 micron) column and methanol: water (90:10v/v) as mobile phase, at a flow rate of 1 ml/min (milliliter per minute) using UV detection at 240nm. The retention time for Ritonavir was found to be 4.109 min. The method was validated for accuracy, linearity, precision, robustness, LOD, and LOQ. Linearity of Ritonavir was observed as 10-50ppm. (R^2=0.9995). The accuracy was done at 50%, 100%, 150%. Recovery was observed as in a range between 98.2%-102.2% for Ritonavir. The precision studies was done and the %RSD values were less than 2%. Lower values of LOD (0.3246ppm) and LOQ (0.9838ppm) was indicated as good sensitivity of the method. This shows that the developed method is simple, precise, accurate and robust for estimation of Ritonavir in tablet dosage form. **Conclusions:** The developed method was found to be simple, selective, sensitive, accurate and repeatable for analysis of Ritonavir in bulk and pharmaceutical dosage form.

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Introduction:

Ritonavir (RTV) is a selective, competitive and reversible inhibitor of the human immunodeficiency virus (HIV) protease enzyme. Chemically it is (5S,8S,10S,11S)-10hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)4-thiazolyl]-3,6-dioxo-8,11- bis (phenylmethyl)-2,4,7,12tetraazatridecan-13-oic acid 5- thiazolyl methyl ester [Figure 1]. It is

widely used in the treatment against the acquired immune deficiency syndrome (AIDS) and prescribed in combination with other antiretroviral drugs as a booster. RITONAVIR is official in Indian Pharmacopeia (IP),[1] British Pharmacopeia,[2] and United States Pharmacopeia (USP).[3] Ritonavir is a selective, competitive inhibitor of liver enzyme Cytochrome P450 (CYP3A)[4] which help to increase the bioavailability of other Protease inhibitors like Atazanavir or Lopinavir in dual protease therapy. Many high performance liquid chromatography (HPLC) methods are reported in biological samples like blood plasma[5,6] and serum,[7] cells[8] and hair.[9] A high performance thin layer chromatography (HPTLC) method is reported in simultaneous estimation of RTV with Lopinavir [10] in capsule dosage form. From the extensive literature review, no analytical methods are reported for the tablet dosage form. The challenges for the quality control and bioavailability of RTV in generics produced in India inspired the authors to develop simple methods for quantification of RTV in bulk and stability study in UV – Visible Spectrophotometer. The developed methods were validated according to International conference of harmonization (ICH) guidelines [11].

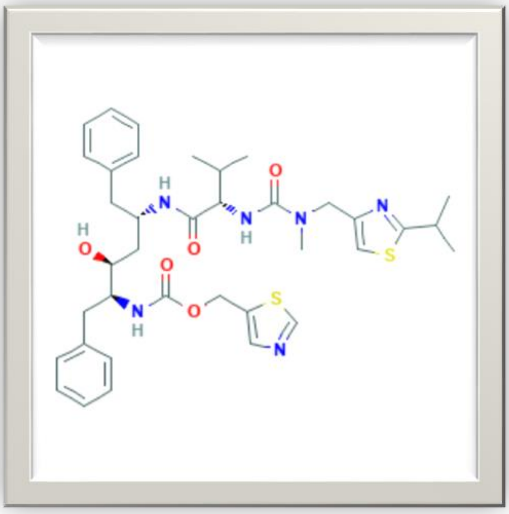

Fig 1: Structure of Ritonavir

EXPERIMENTAL SECTION

Materials and Methods:

Instrumentation: A binary gradient system of 3000 series HPLC of analytical technologies ltd, was used for analysis.

Chemicals: A standard Ritonavir was procured from mylan pharmaceuticals pvt. Ltd. And other chemicals like methanol, water, of HPLC grade were purchased from LobaChemie, Mumbai.

Method development:

Solubility: As per literature review and solubility test the standard Ritonavir was found better soluble in methanol.

Method: As per the trials done on combination of methanol water system as mobile phase, I'd got better results at 240 nm wavelength and 90:10 ratio of mobile phase as methanol: water.

Method validation: A developed method was validated by some methods like Linearity, Accuracy, Precision, % Recovery, Assay, Limit of detection (LOD), Limit of Quantification (LOQ), Robustness, and Ruggedness as per ICH Guidelines.

Preparation of stock solution: 10mg of standard drug Ritonavir was dissolved in 10 ml of mobile phase solution as Methanol: water, it gives 1000ppm stock solution.

Preparation of test solution: For preparation of test solutions of 10, 20, 30, 40 & 50 ppm dilutions, I've taken 1, 2, 3, 4 & 5 ml of solution from the stock solution of 1000 ppm and make up the volume up to 10 ml with the diluent solution respectively.

RESULTS AND DISCUSSION

Validation Parameters for method development:

Linearity: To perform linearity I'd prepared dilutions of 10, 20, 30, 40&50 ppm concentration, and validated it on HPLC as per ICH guidelines.

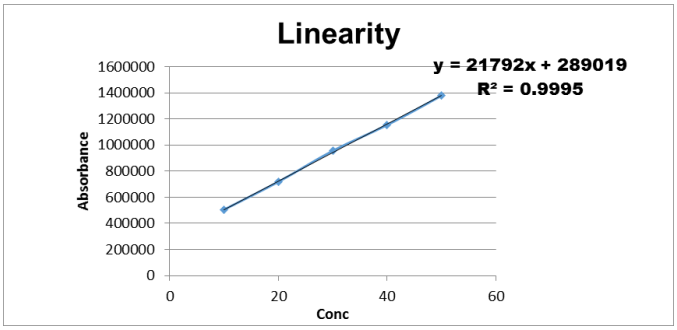

Fig2: Calibration curve of Ritonavir

Accuracy:

The accuracy of method was determined by recovery tests. So, a well-known amount of concentration of working standard was added to fixed concentration of the pre-examined tablet solution. And the percent recovery was calculated by matching the area before and after addition of working standard. The recovery studies were done 3 times. This standard addition method was performed at 50%, 100%, 150% concentrations and the percentage recovery was calculated. The percent

recovery was within the range of 98.2 to 102.2 for Ritonavir.

Table 1- Accuracy study of Ritonavir

Sr. No.	% Composition	Sample Amount		Area of Sample	Amount Recovered in ppm	% Recovery	% Mean Recovery	% SD
		Sample Amount in ppm	Amount Added in ppm					
1	50% Recovery	20 20 20	10 10 10	955723 955532 955321	30.0128 30.0188 30.0254	100.9574325 100.9374562 100.9153807	100.94	0.0918543
2	100% Recovery	20 20 20	20 20 20	1151239 1151957 1151654	40.1039 40.0789 40.0895	100.7408662 100.8030721 100.7768209	100.77	0.11247
3	150% Recovery	20 20 20	30 30 30	1375886 1375684 1375351	50.0833 50.0833 50.1028	100.8336934 100.8190364 100.7948741	100.81	0.090661

Precision:

A standard solution containing 30 ppm of Ritonavir were analyzed three times on the same day and different day, and the % RSD was calculated. The results are given in table.

Table 2- Precision study of Ritonavir

Interday Precision		Intra-day Precision	
Day 1	Area	Morning	Area
	956130		956130
	957995		957995
	955142		955142
Day 2	956026	Evening	955723
	955523		956404
	956334		955671
Mean	956334	Mean	956177.5
SD	899.2991	SD	902.98
% RSD	0.09405%	%RSD	0.094436%

Robustness:

By doing a small thoughtful changes in chromatographic conditions likewise, a change in wavelength (±2 units) and flow rate (±2 units) were studied, to observe the robustness of the method. The results were found less than 2 of tailing factor of the developed RP-HPLC method for the analysis of Ritonavir. The results are given in table.

Table 3- Robustness study of Ritonavir

Level	Ritonavir	
	Retention time	Tailing factor
Change in	Flow rate (ml/min)	
-2(0.8ml)	5.083	1.09
0(1ml)	4.097	1.11
+2(1.2ml)	3.433	1.06
Change in	Wavelength (nm)	
-2(238nm)	4.073	1.11
0(240nm)	4.097	1.11
+2(242nm)	4.098	1.11

Assay:

The assay for Ritonavir tablet was performed and the % purity were calculated as follows.

%Assay =

(Sample area ÷ Standard area) × (Weight of standard ÷ Dilution of standard) × (Dilution of sample ÷ Weight of sample) × (Purity of drug ÷ 100) × (Weight of tablet ÷ Labelled claim) ×100

= (955846÷956130) × (10÷30) × (30÷10) × (100÷100) × (100÷100) × 100

=99.97%

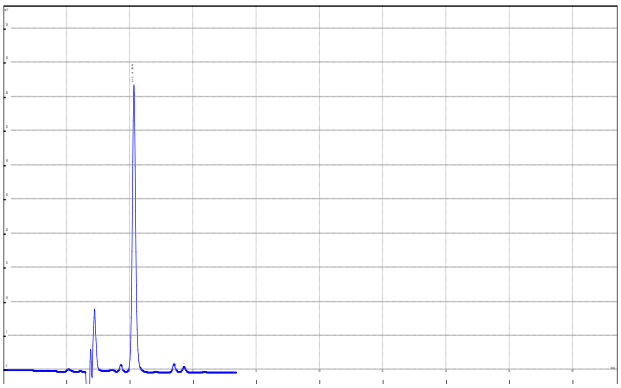

Fig 3: Chromatogram showing assay of standard injection

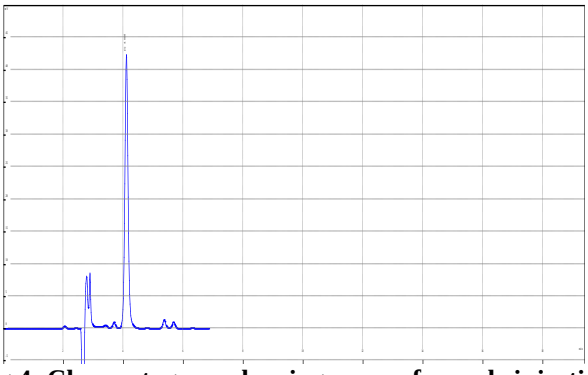

Fig 4: Chromatogram showing assay of sample injection

The % purity of Ritonavir and in pharmaceutical dosage form was found to be 99.97%

Limit of detection (LOD) and limit of quantitation (LOQ)

From linearity data, the LOD and LOQ was calculated by using formula LOD = 3.3×standard deviation ÷ Slope and LOQ = 10×standard deviation ÷ Slope. Where, standard deviation is of y intercept of linearity equations and slope is of calibration curve of the analyte. LOD and LOQ were found to be 0.3246 µg/mL and 0.9838µg/mL respectively.

Conclusion

The developed method was found to be simple, selective, sensitive, accurate and repeatable for analysis of Ritonavir in bulk and pharmaceutical dosage form.

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