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Simple validated isocratic RP – LC method for estimation of Ritonavir in bulk and tablet dosage form

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ABSTRACT

A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of Ritonavir in tablet dosage form. The estimation was carried out on HiQSil C18 (25cm x 4.60 mm, particle size 5µm) column with a mixture of methanol: acetonitrile: water in the ratio of 87:10:3(v/v/v) as mobile phase. UV detection was performed at 240 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 3.6 min. for Ritonavir and total run time was 10 min. at a flow rate of 1.0 ml/min. The calibration curve was linear over the concentration range of 25 - 200 µg/ml for Ritonavir. The LOD and LOQ values were found to be 0.286µg/ml and 0.858µg/ml. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the estimation of Ritonavir in tablet dosage form.

Keywords: Ritonavir, RP-HPLC, Validation, tablet dosage form

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)-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- thiazolyl methyl ester (Fig. 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. RTV is official in IP¹, BP² and USP³. Ritonavir is a selective, competitive inhibitor of liver enzyme CYP3A⁴ which helps in increase in

Preparation of stock solution, working solution and standard calibration curve

A stock solution of 1000µg/ml of RTV was prepared in Methanol. The stock solution was diluted further with methanol to obtain working solutions with concentrations of 200, 150, 100, 50 and 25µg/ml. The prepared samples were also filtered through 0.45µm nylon filter membrane before injection. The injection volume was 10µl. The standard calibration curve was plotted by AUC Vs Concentration at 240nm.

Sample Preparation

Commercially marketed tablets of RTV, Ritomune (100mg, Cipla) and Viriton (100mg, Ranbaxy) were purchased. The samples were prepared by extraction with methanol. Twenty tablets were weighed carefully, average weight was calculated and equivalent amount of solid content was weighed accurately. Weighed amount was dissolved in methanol to prepare the sample solution of concentration 1000µg/ml. Desired dilution of sample solution was prepared. The sample was filtered through Whatman filter paper No 41, then through 0.45µm nylon membrane filter before injection.

Method Validation

The developed method was validated according to ICH guidelines¹⁰. The validation parameters were linearity, specificity, accuracy, precision, Limit of detection (LOD), Limit of Quantification (LOQ). Intra-day and Inter-day precision values were estimated by assaying the pharmaceutical dosage form containing three different concentrations of RTV six times on the same day and on three different days. Accuracy was determined by recovery study by standard addition method. The standard was added to a predetermined concentration at 25%, 50% and 100% level. The LOD and LOQ was determined by using equation (1) and (2) respectively

$$\text{LOD} = 3.3 \sigma / S \text{ ----- (1)}$$

$$\text{LOQ} = 10 \sigma / S \text{ ----- (2)}$$

Where 'σ' is the standard deviation of y-intercept and 'S' is the slope of calibration curve.

RESULT AND DISCUSSION**Method development**

To develop an efficient and reproducible method for assay of RTV in pharmaceutical dosage form many solvent system combinations were tried, like methanol, water, acetonitrile and tetrahydrofuran. Generally the retention time of PIs is dependent on pH of the solvent system¹¹. To avoid the tedious preparation and maintenance of buffers, the mixture of organic solvents and water were chosen. So the solvent methanol, acetonitrile and water in the ratio 87:10:3 were selected, which is easy and cost-effective. Isocratic mode was preferred to gradient elution as it requires long re-equilibrium time, perfect mixing. The analysis was done at normal room temperature. The method was found to be accurate, precise and specific at the wavelength 240nm. Using these chromatographic conditions, the retention time of RTV was found to be 3.6 ± 0.04 minutes.

Calibration curve and Linearity

A five point calibration standard curve of RTV was plotted ranging from 25 µg/ml - 200µg/ml (Injection volume = 10 µl per injection). The calibration curve was linear over the tested range and the coefficient of correlation was found to be 0.999 at 240nm (Figure - 2).

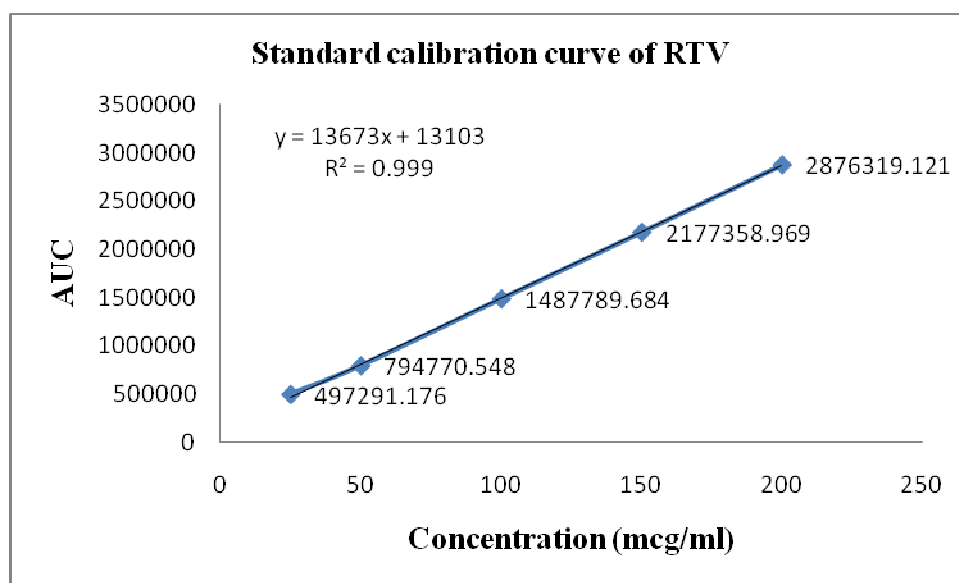


Figure 2 – Standard calibration curve of Rtonavir

Assay of tablet dosage form

The amount of RTV was calculated from the standard calibration curve. The results are reported in table – 1.

Table -1. Assay of Tablet Formulation

Name of the formulation	Label claim (mg)	Amount found (%) MEAN*	R.S.D
Viriton	100	100.01	0.609
Ritomune	100	99.485	0.293

*Mean of six determinations

Recovery Study

The recovery obtained in Viriton was 99.584 ± 1.207 and for Ritomune, the recovery was 99.953 ± 1.393 (Table - 2). Low value of relative standard deviation (< 2) shows the accuracy of the developed method.

Table 2: Accuracy of the method (Recovery study)

Name of the formulation	Amount of sample taken ($\mu\text{g/ml}$)	Amount of standard added ($\mu\text{g/ml}$)	Amount found (%)	Mean* \pm R.S.D
Viriton	100	25	98.88	99.584 ± 1.207
	100	50	100.973	
	100	100	98.9	
Ritomune	100	25	98.62	99.953 ± 1.393
	100	50	99.84	
	100	100	101.4	

*Mean of three determinations

Precision

The Precision of the method was assessed by mean \pm R.S.D of intra-day and inter-day assay (Table - 3). The intra-day assay shows relative standard deviation of 0.157 and 0.357 for Viriton and Ritomune respectively. The inter-day assay shows relative standard deviation of 0.347 and

0.378 for Viriton and Ritomune respectively. The low values of relative standard deviation show the repeatability and reproducibility of the method.

Table 3: Precision of the method

Name of the formulation	Intra-day precision (n =6) Mean* \pm S.D	Inter-day precision (n = 3) Mean* \pm R.S.D
Viriton	99.653 \pm 0.157	99.03 \pm 0.342
Ritomune	99.42 \pm 0.357	99.59 \pm 0.378

*Mean of six determinations

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD value was found to be 0.286 μ g/ml and the LOQ value was found to be 0.858 μ g/ml.

Selectivity and Specificity

The method was found to be selective by varying the different experimental conditions. By varying the experimental conditions like slight change in composition of solvent system, flow rate, change in the wavelength (\pm 5nm), the retention time, shape of the chromatogram were varied. So this is a selective method of analysis of ATV in pharmaceutical dosage forms. The method is very much specific as there was no interference of excipients. The chromatograms of the standard drug and that of the tablet sample were compared; both have the same retention time. (Figure 3,4 and 5).

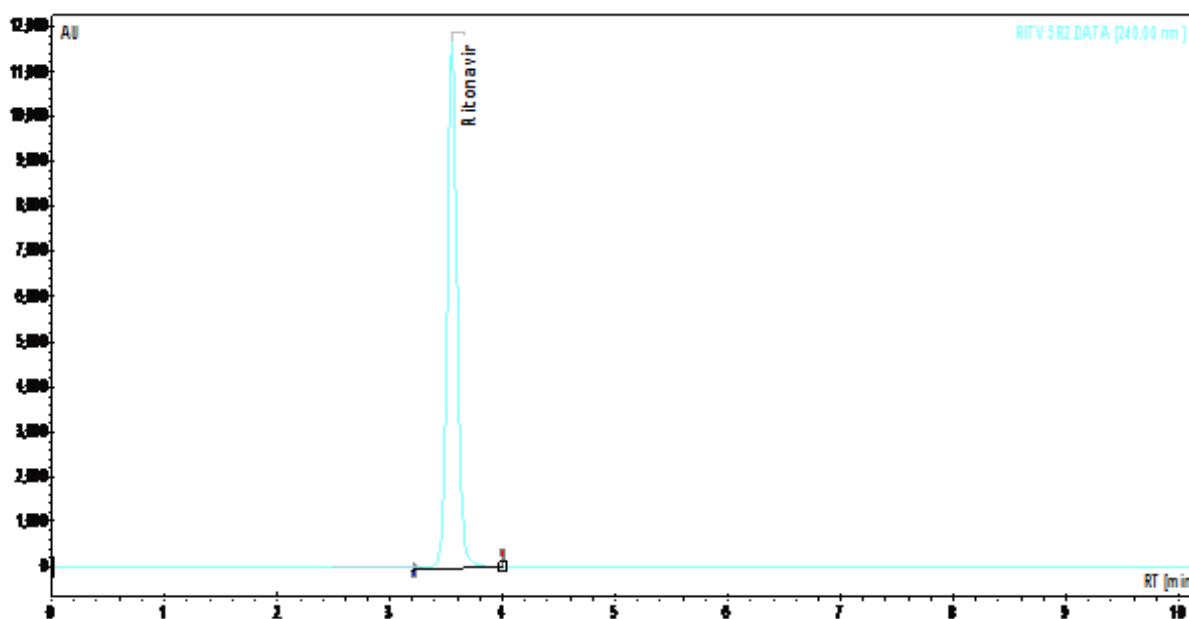


Figure 3 – Representative chromatogram of Ritonavir at 240nm

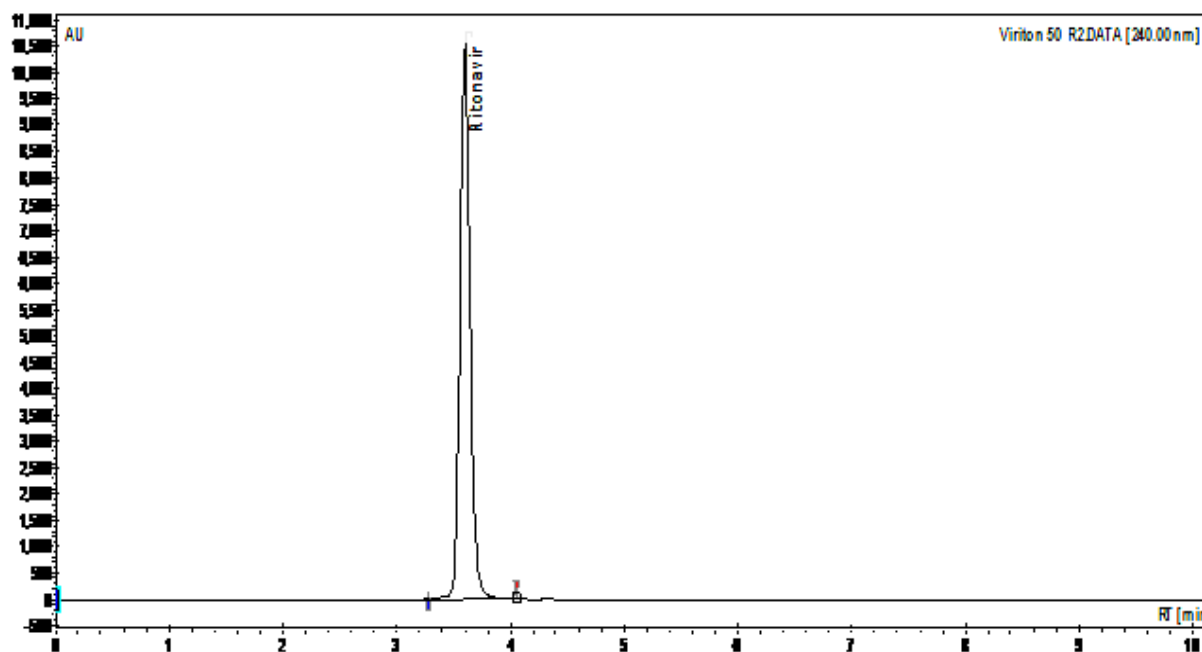


Figure 4 – Representative chromatogram of Viriton at 240nm

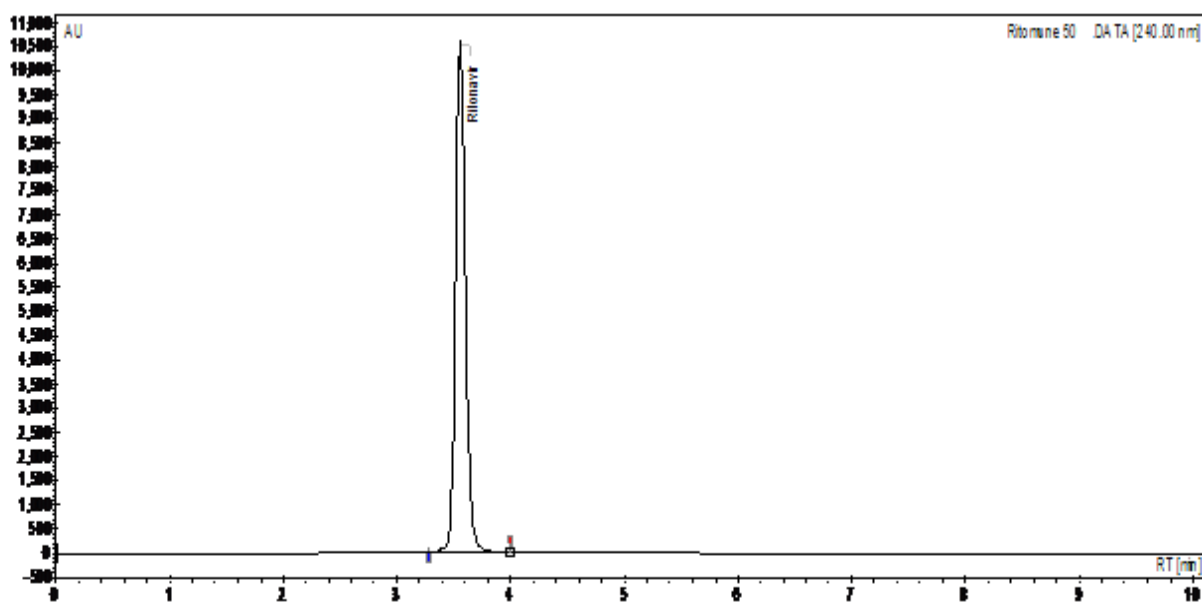


Figure 5 – Representative chromatogram of Ritomune at 240nm

System suitability parameters

The developed method also checked for the system suitability parameters. The retention time, asymmetric factor and number of theoretical plates found are in accordance with the official monograph. The parameters are given in table 4. The parameters justify that the method is good for separation of RTV.

Table 4 – System suitability parameters

Parameters	
Retention time, R_t	3.6 ± 0.04
Asymmetric factor, T_f	1.09 ± 0.02
Number of theoretical plates, N	7493.10

CONCLUSIONS

A simple, precise, selective and sensitive isocratic HPLC assay method with PDA detection for RTV in pharmaceutical dosage form has been developed and validated. The method will be extensively used for evaluation of bioavailability of RTV in in-vitro study. An extended study for the in – vitro bioavailability in the form of enhancement and decrease of therapeutic efficiency in combination with Atazanavir and/or Lopinavir may be an important subject for study in the evaluation of 2nd line anti HIV therapy.

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