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A Simple UV Spectroscopic Method for the Determination of Ritonavir in Bulk and Tablets

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Abstract

A simple, economic, accurate UV method was developed for the estimation of Ritonavir (RV) in bulk and tablet dosage form. Water and methanol in the ratio of 50:50 v/v was used as a diluent to dissolve RV. The drug mixture was sonicated for 2 mins for the enhanced solubility. The absorptions were observed at 240.0 nm, which was selected for the further analysis of RT in bulk and its tablet dosage forms. The proposed method was validated according to ICH guidelines. The method showed high sensitivity with linearity range from 10 to 80 µg/ml (r^2=0.999) at 240.0 nm. The limit of detection (LOD) was found to be 50 µg/ml and the limit of quantization (LOQ) was determined as the lowest concentration was found to be 200 µg/ml. The reports expressed that the proposed method was found to be simple, precise, accurate and rapid for the estimation of RT in bulk and tablet dosage form using UV spectroscopy.
Keywords: Ritonavir, methanol, water, UV spectroscopy, ICH guidelines

Contents

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1. Introduction

Ritonavir (RV) is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Ritonavir is frequently prescribed with Highly Active Anti-Retroviral Therapy, not for its antiretroviral action, but as it inhibits the same host enzyme that metabolizes other protease inhibitors. This inhibition leads to higher plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy. The lower than therapeutic doses of ritonavir are commonly given in combination with agents such as Lopinavir, Indinavir, or Amprenavir to reduce the risk of resistance by increasing the time of drug exposure [1].

Combination therapy with the HIV protease inhibitors Lopinavir and ritonavir (Sustained release capsule with combination of Lopinavir 133.3 mg and ritonavir 33.3 mg is available in market by brand name kaletra®) has been shown to be effective against drug-resistant HIV-13. These agents are metabolized by cytochrome P-450 (CYP) 3A in the liver [2-4]. When Lopinavir is administered with ritonavir as kaletra®, ritonavir inhibits the CYP 3A-mediated metabolism of Lopinavir, thereby providing increased plasma levels of Lopinavir.

From the literature survey, it was found that RV estimated by analytical methods such as spectrophotometric methods [5-7], reversed-phase high-performance liquid chromatographic (RP-HPLC) method [8] and HPTLC method [9]. Hence, the aim of this study was to develop and validate a simple UV method, to quantify RV in pure form and pharmaceutical formulation (tablets). The proposed method was validated according to ICH guidelines [10]. The validated method was applied to the analysis of tablets containing ritonavir (100 mg). The chemical structure of Ritonavir is shown in figure 1.

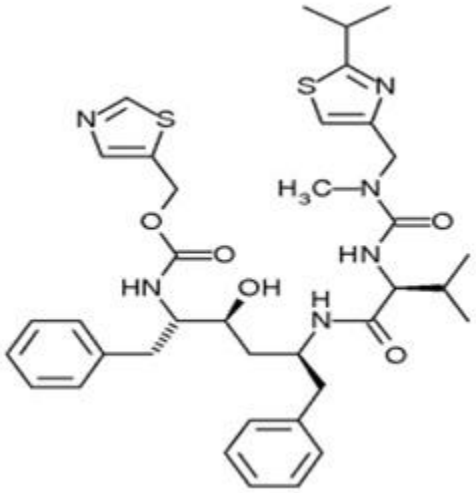

Figure 1. Chemical structure of Ritonavir

2. Materials and Methods

Instruments and reagents

An analytically pure sample of RV was procured as gift sample from Matrix laboratories (Hyderabad, India). Analytical grade methanol was used as solvent for dilution. Distilled water was prepared in house. A PG Instruments T-60 UV/VIS spectrophotometer was used with 1 cm matched quartz cell. Tablet formulation [Ritomune 100 mg Cipla, India] was procured from a local pharmacy with labeled amount 100 mg per tablet. Water: methanol in the ratio of 50:50 v/v was used as a diluent to dissolve RV.

Preparation of working standard drug solution

The standard RV (50 mg) was weighed accurately and transferred to volumetric flask (50 ml). It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. 30 µg/ml solution was prepared from the stock solution which was used as working standard.

Analysis of marketed formulations

For the estimation of Ritonavir in tablets formulations, 20 tablets were weighed and triturate to fine powder. Tablet powder equivalent to 50 mg of Ritonavir was weighed and transfer into 50 ml volumetric flask than dissolved in diluent. It was kept for sonication for 3 min; this was filtered through Whatman filter paper No. 41 and then final dilution was made with diluent to get the final stock solution of 1000 µg/ml. From this stock solution, various dilutions of the sample solution were prepared and analysed.

Validation

Method validation was performed in terms of specificity and selectivity, precision and accuracy, linearity, LOD & LOQ.

Linearity and range

Calibration standards of RV, covering the range 10-80 µg/ml were prepared with the suitable dilution made from RV stock solution. The calibration curves were obtained by plotting the intensity of absorbance against of concentration of RV. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

Specificity and selectivity

The interference from endogenous compounds was investigated by the analysis of tablets of various concentrations.

Precision

The intra & inter-day precision was evaluated by analyzing six sample solutions (n = 6), at the final concentration of analyses (30 µg/ml) of RV. The RV concentrations were determined and the relative standard deviations (RSD) were calculated.

Accuracy

RV reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (15, 30 and 45 µg/ml of ritonavir). At each level, samples were prepared in triplicate and the recovery percentage was determined.

Detection and quantitation limits

Limit of detection LOD and limit of quantification LOQ were calculated by using the standard deviation from the precision and the slope of linearity.

3. Results and Discussion

RV has the zero order absorbance spectra maxima (figure 2 and 3) at 240.0 nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 10-80 µg/ml with correlation coefficient (r^2) was found to be higher than 0.999 and the linearity curve was shown in figure 4. Recovery studies were carried out at three different levels i.e. 50 %, 100 %, and 150 % by adding the pure drug to the previously analysed tablet powder sample. Percentage recovery for Ritonavir was determined by all the methods and they were found to be under acceptance criteria which are 98% to 102 % according to ICH guidelines [9]. The results of accuracy were in table 2. The percentage recovery value indicates noninterference from excipients used in formulation. The precision was carried out as described in method and the results were presented in table 1. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision values; hence the developed method can be used to analyze the RV in tablet formulation. The mean assay of the precision value is 100%. The LOD determined as the amount drug was found to be 50 µg/ml and the LOQ was determined as the lowest concentration was found to be 200 µg/ml in formulation. The summary of all the optical characterizes were shown in table 2.

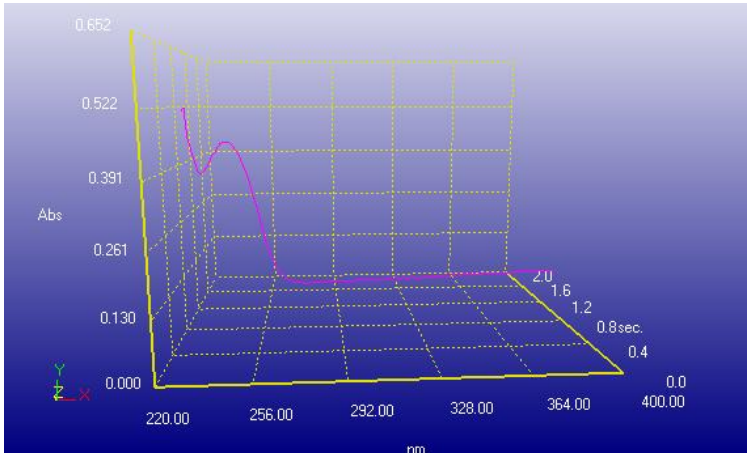

Figure 2. λ_{max} (3-D view) curve of Ritonavir

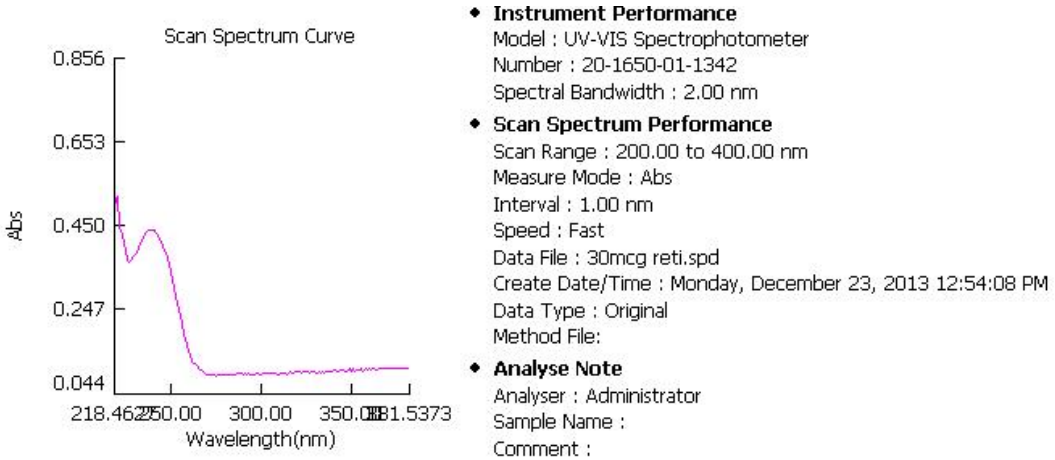

Figure 3. λ_{max} (2D view) curve of Ritonavir

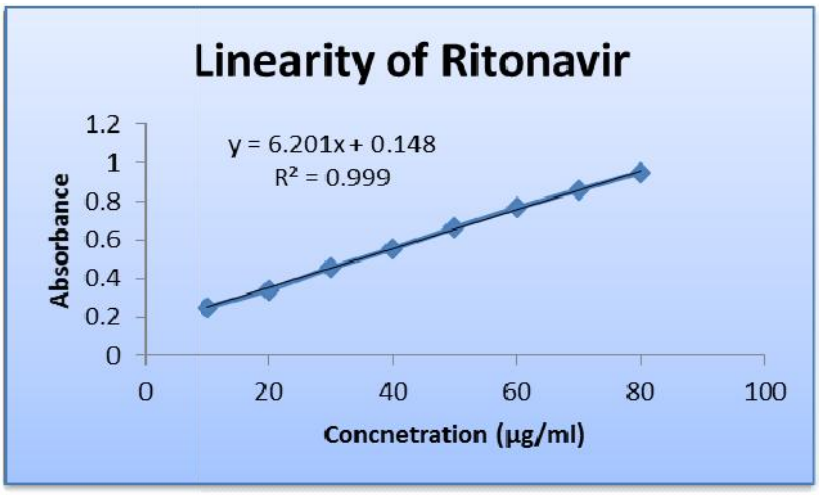

Figure 4. Linearity curve of Ritonavir

Table 1. Results of Precision

Sample No.	Sample Abs	% Assay
1	0.489	100.59
2	0.479	98.53
3	0.488	100.38
4	0.489	100.59
5	0.48	98.74
6	0.489	100.59
	Average Assay	100
	STD	0.99
	% RSD	**0.99**

Table 2. Summary of Optical characteristics and Other Parameters

S No.	Parameters	Results
1	Absorption Maxima (nm)	240
2	Beer's-Lambert's range (µg/ml)	10-80
3	Regression equation (y)*	y = 6.201x + 0.148
4	Slope (b)	6.201
5	Intercept (a)	0.148
6	Correlation coefficient (r^2)	0.999
7	Sandell's sensitivity (µg/ cm^2-0.001 absorbance units)	0.0755
8	Intraday precision (% RSD)**	0.99
9	Interday precision (% RSD)**	0.98
10	Accuracy (% mean recovery)	100
11	Limit of detection (µg / ml)	50
12	Limit of quantification (µg / ml)	200
13	Assay of tablets (%Purity)	100

*y = a + bx; when x is the concentration in mg/ml and y is absorbance unit.
**Average of six determinations.

4. Conclusion

The most striking features of the method was its simplicity and rapidity, non- requiring consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC or other procedures. It can be concluded that the proposed methods was fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed method can be easily applied for the routine Quality Control analysis of RV in pharmaceutical preparations.

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