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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF RITONAVIR IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Ritonavir in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point which is done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Ritonavir in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, soluble in isopropanol and practically insoluble in water. So, the analytical grade methanol is used as a diluent in method. The melting point of Ritonavir was found to be 130-131˚C (uncorrected). It showed absorption maxima 252 nm in analytical grade methanol. On the basis of absorption spectrum, the working concentration was set on 50µg/ml (PPM). The linearity was observed between 10-90 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 98.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.99% and for Ruggedness is 0.57%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Ritonavir in tablet dosage form. The method could be considered for the determination of Ritonavir in quality control laboratories.

KEYWORDS: Ritonavir, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Ritonavir is chemically designated as 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, [5S- (5R*,8R*,10R*,11R*)]. Ritonavir is an inhibitor of HIV protease with activity against the Human Immunodeficiency Virus3-5. Ritonavir is a white-to light tan powder.[1,2] It has a bitter metallic taste and freely soluble in methanol and ethanol, soluble in isopropanol and practically insoluble in water.[3] Its molecular weight is 720.95. Its molecular formula is $C_{37}H_{48}N_6O_5S_2$. 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.[4]

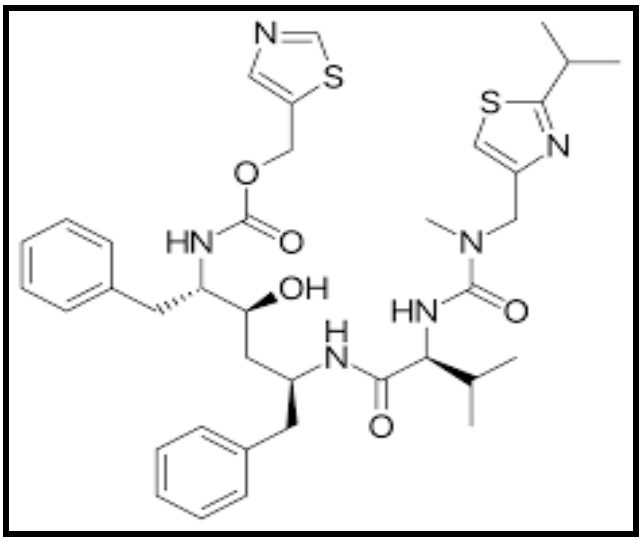

Fig. 1: Chemical Structure of Ritonavir.

From the literature survey, it was found that Ritonavir estimated by analytical methods such as

spectrophotometric method on capsule form[5], absorption ration UV method[6], UV determination in combination with atazanavir[7], reversed-phase high-performance liquid chromatographic (RP-HPLC) method[8-10] and HPTLC method.[11] Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity, and low cost. Some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Ritonavir alone in tablet dosage form.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.
Melting point apparatus of Equiptronics were used.

- **Materials**

Ritonavir was obtained as a gift sample. Ritonavir tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method development

A. Determination of λ max (50 PPM)[12, 13, 14]

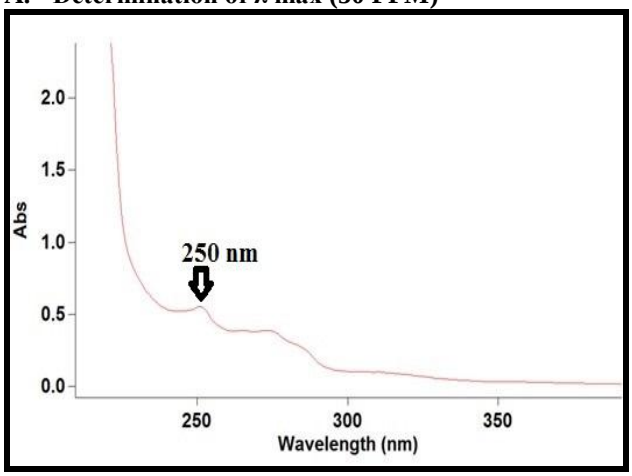

Fig. 2: Calibration Curve.

20 mg weighed amount of Ritonavir was dissolved into 200 ml of volumetric flask with analytical grade methanol. Pipette out 5 ml and added in 10 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of Working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 20 mg of Ritonavir in 200 ml of analytical grade methanol to get concentration of 100 µg/ml (PPM).

Preparation of Standard solution

Pipette out 5 ml from standard stock solution and diluted up to 10 ml with analytical grade methanol to get concentration of 50 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)
Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[15,16,17]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 20 mg of Ritonavir was accurately weighed and transferred into the 200 ml of volumetric flask, added 150 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 200 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 5 ml of the filtered solution and diluted up to 10 ml with analytical grade methanol. The absorbance was measured at 252 nm. The absorbance was recorded.

Table 1: Absorbance of Dosage Form.

Cipla Pharma Pvt. Ltd. (Ritonavir 100 mg Tablets)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.5384
3	Sample	0.5307

Table 2: Dosage Form Specifications.

Type	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	Ritomune ® - 100 Cipla Pharma Pvt LTD (100mg)	11/2022	11/2024	P 1420567	0.1652	98.57

E. Method of validation[17, 18]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 10 - 90 PPM at 252 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	10 PPM	0.1171
2	30 PPM	0.3231
3	50 PPM	0.5352
4	70 PPM	0.7518
5	90 PPM	0.9714
Correlation coefficient		0.9998 ~ 0.999

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 10 ml of volumetric flask with analytical grade methanol and measuring the absorbance at 252nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75
100	1	0.98	98.00
120	1.2	1.19	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5254
2	Sample Solution 2	0.5305
3	Sample Solution 3	0.5214
4	Sample Solution 4	0.5287
5	Sample Solution 5	0.5352
MEAN		0.5282
SD		0.0052
% RSD		0.9865 ~ 0.99

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5328	0.5333	99.04	0.5679
		0.5337			
2	Analyst 2	0.5356	0.5376	99.84	
		0.5395			

RESULTS

1. Solubility of Ritonavir

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Methanol, Ethanol	Freely Soluble
2	Isopropanol	soluble
3	Water	Practically Insoluble

2. Melting point of Ritonavir

The melting point of Ritonavir was found to be 130-131˚C (uncorrected).

3. Results for linearity for assay method of Ritonavir

The linearity of method was determined at concentration level ranging from 10 to 90 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9998 ~ 0.999.**

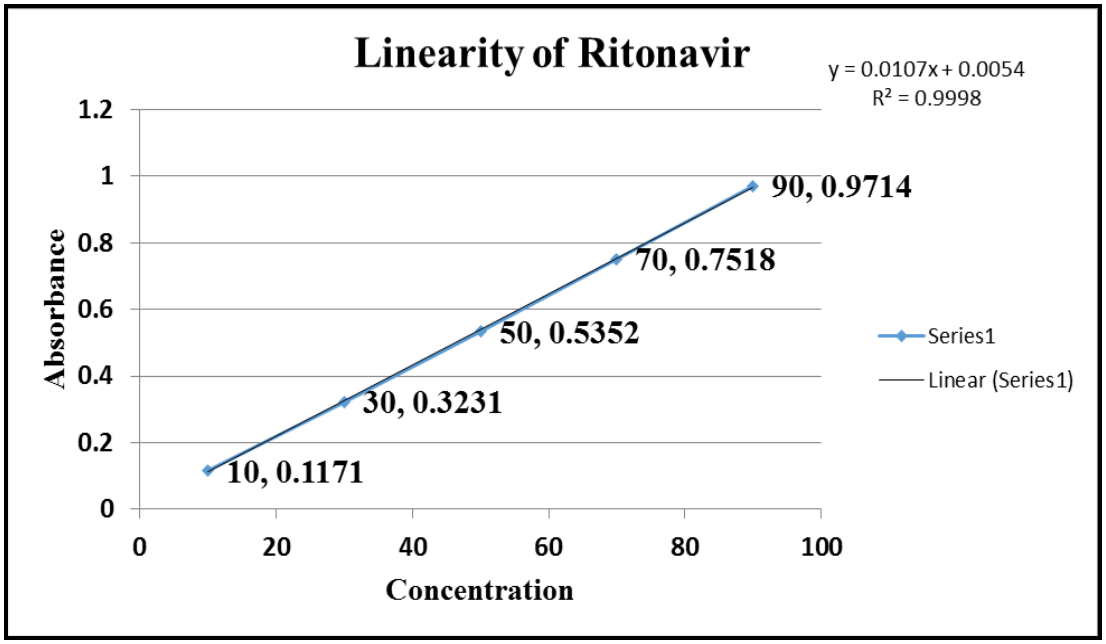

Fig. 3: Ritonavir Standard Curve.

4. Results for accuracy for assay method of Ritonavir

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Ritonavir

The % RSD for different sample of precision was found to be 0.9865 ~ 0.99 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Ritonavir

The %RSD for different sample of ruggedness was found to be 0.5679 ~ 0.57 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Ritonavir in tablet form has been developed. From the spectrum of Ritonavir, it was found that the maximum absorbance was 252 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 10-90 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Ritonavir in solid dosage forms. Hence, the method could be considered for the determination of Ritonavir in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. HPLC - High Performance Liquid Chromatography
4. UV - Ultra violet
5. MS - Mass Spectroscopy
6. LC - Liquid Chromatography
7. ICH - International Council for Harmonization
8. RSD - Relative Standard Deviation
9. SD - Standard Deviation
10. Qty - Quantity
11. °C - Degree Celsius
12. M.D. - Manufacturing Date
13. E.D. - Expiry Date
14. µg/ml - Microgram per milliliter
15. Avg - Average

16. Wt - Weight
17. g - gm
18. DMF – Dimethylformamide

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