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Original Article

Development and Validation of New Analytical Methods for the Estimation of Ritonavir in Bulk and Pharmaceutical Dosage Forms

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A R T I C L E   I N F O

A B S T R A C T

Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs. In the present investigation potent drug namely Ritonavir only few analytical methods were reported and hence there is wide scope for the development of new analytical methods for its quantitative analysis. All the developed methods are complimentary to each other and the proposed methods can be used as alternative methods to reported ones thereby providing a wide choice for routine determination of the Ritonavir in bulk and pharmaceutical preparation. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ritonavir in bulk and pharmaceutical dosage forms. The results expressed for HPLC is promising. In addition to positive requirements for analytical methods, the striking advantage of all the presently developed methods is that they are economical. And method is validated in terms of accuracy, precision, linearity, and limit of detection, limit of quantitation, and robustness. This method can be used for the routine determination of Ritonavir in bulk and pharmaceutical dosage forms.

Keywords: Ritonavir, pharmaceutical preparation, RP-HPLC, limit of quantitation.

1. INTRODUCTION

Drugs and pharmaceuticals are chemicals or like substance, which are of organic, inorganic or other origin. Whatever may be origin we use some property of the medicinal agent to measure them quantitatively or qualitatively. Qualitative analysis is the identification of elements, species or compounds present in a sample[1]. Quantitative analysis is the determination of the absolute or relative amounts of

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1436

elements, species or compounds present in a sample. The aim of the study is develop and validate new analytical methods for the estimation of ritonavir in bulk and pharmaceutical dosage forms[2,3].

2. MATERIALS AND METHOD

Drug profile[4,5]

Category: Antiretroviral agent

Chemical name:1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate.

Molecular formula:$C_{37}H_{48}N_6O_5S_2$

Molecular weight:720.944

Description: A white to off-white powder

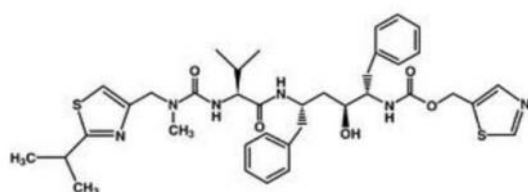**Solubility:** Practically insoluble in water, freely soluble in Methanol, Ethanol, Acetonitrile

Fig 1: Ritonavir

Methods

Method development, Validation and Forced degradation studies of Ritonavir by RP-HPLC[6]

Trail-1

Column: INERTSIL C18 250 x 4.6mm

Injection Volume: 20 µl

Diluent: Methanol: Water (70:30)

Absorbance: 239 nm

Observation: Here the Retention time is too long i.e. 13 and further mobile phase. Polarity should be increased.

Flow rate	Methanol	Water
1.00 ml/min	50	50

Trail-2

Column: INERTSIL C18 250 x 4.6 mm

Injection Volume: 20 µl

Diluent: Methanol: water (70:30)

Absorbance: 239 nm

Observation: Theoretical plates < 2000, Tailing factor was found to be > 2.

Flow rate	Methanol	Water
1.00 ml/min	70	30

Preparation of Ritonavir Tablets[7]

Ritonavir : 100 mg

Cospovidone : 5mg

Talc : 5mg

Magnesium stearate:5mg

Micro crystalline cellulose:120 mg

Method: Direct compression method

Preparation of Sample solution

Ten tablets were accurately weighed and crushed. Powder equivalent to 2 tablets was taken and transferred into 200 ml volumetric flask, followed by the addition of 170 ml of diluent, sonicated and diluted upto the mark. From this 4 ml was taken and diluted to 25 ml with the diluent, filtered through 0.45 µ nylon syringe filter. 20 µL of the standard, sample solution (160µg/ml) was injected into the chromatographic system, area for the Ritonavir peak was measured and the % Assay was calculated by using the formulae[8, 9].

3. RESULTS & DISCUSSIONS

Method development, Validation and Forced degradation studies of Ritonavir by RP-HPLC:

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a simple, rapid, precise, accurate and sensitive HPLC method for the analysis of Ritonavir in bulk and its pharmaceutical dosage form by using solvent system of ACN: OPA in the ratio 55:45 and Zodiac C18, 150mm x 4.6mm, 5µm stationary phase. The chromatographic condition is set at flow rate of 1ml/min with PDA detector at 239 nm. As per ICH requirements validation studies are carried out by using freshly prepared solutions[10]. Optical characteristics were given in table no.1

Table 1: Validation parameters of Ritonavir by HPLC

PARAMETER	ACCEPTANCE CRITERIA	RITONAVIR
Linearity Range Correlation Coefficient	Correlation coefficient $r^2 >$ 0.999 or	8.0 – 240µg/ml $r^2 = 0.99982$
LOD	S/N > 2 or 3	0.172064 µg/ml
LOQ	S/N > 10	0.521407 µg/ml
System Precision	RSD < 2%	%RSD = 0.456
Intermediate Precision	RSD < 2%	%RSD = 0.385
Method precision	RSD < 2%	%RSD = 0.298
Accuracy	Recovery 98- 102% (individual)	% recovery= 98.08-101.9
Specificity	1) No interference from blank, placebo and other degradation products with the main peak.	No interference.
	2) Purity angle < threshold angle	Peak pure
Solution Stability	> 12 hour	Stable up to 24 hour %RSD = 0.55
Robustness	RSD NMT 2% in modified condition Flow minus Flow plus Organic plus Organic minus Wavelength plus Wavelength minus	Complies %RSD= 0.21 %RSD= 0.06 %RSD=0.46 %RSD=0.38 %RSD= 0.094 %RSD= 0.107

Effect of solvents on ᵐᵃˣ of Ritonavir:

The solubility of Ritonavir was determined as per Indian pharmacopeia. As the drug Ritonavir is insoluble in all the solvents initially drug was dissolved in methanol and further dilutions were made with corresponding solvents[11]. And the concentration prepared was 10 µg/ml. ₘₐₓ of Ritonavir in various solvents were tabulated.

Table 2: ₘₐₓ ₒf **Ritonavir in various solvents**

S.No.	Solvent	ₘₐₓ (nm)
1.	Methanol	239
2.	Water	240
3.	0.1N HCl	241.5
4.	0.1N NaOH	240.5

Method development and Validation of Ritonavir by Titrimetry:

A simple precise, rapid accurate and sensitive non-aqueous titration method was developed for quantitative determination of Ritonavirin pharmaceutical dosage form.The titration was carried out using standardized 0.1 N perchloric acid. The proposed method was found to be precise with % RSD =1.86 which is less than 2 (n = 6). The method showed strict linearity (r^2 = 0.999) of 100 mg of drug substance weight. The percentage recovery of Ritonavir in the optimized method was between 100.03 % to 101.85 %. The method is also found to be rugged when checked by different analysts and using different lots of reagents and different makes of titrators[12].

Method development, Validation and Forced degradation studies Of Ritonavir by UV Spectroscopy:

An effort has been made to identify a simple, precise, rapid, specific and accurate method for the estimation of Ritonavir in bulk and in formulation.The solubility of Ritonavir was determined as per Indian pharmacopoeia. Number of solvents tried include Distilled water, Methanol, Ethanol, Acetonitrile, Isopropanol. It is insoluble in Distilled water. It is freely soluble in Methanol and ethanol. It is very slightly soluble in 0.1N HCl. By considering thecost of Methanol, solubility of the drug was tried with 0.1NHCl and to increase the solubility intially drug was dissolved in methanol, further dilutions were made with 0.1N HCl. The solution was scanned in UV region in the wavelength range from 200-400 nm against solvent as the blank. From the spectrum of Ritonavir the wavelength maxima was found to be 243.5 nm. Different aliquots of Ritonavir were prepared in the concentration range of 10-30µg/ml with the solvent. The absorbances of solution were measured at 243.5 nm. The calibration curve was plotted using concentration Vs absorbance. The correlation coefficient value for the calibration graph was found to be 0.999. The slope and intercept value was found to be 0.009 and 0.083 respectively. It indicates that the concentration of Ritonavir has a good linearity in the range 10-50 µg/ml.

LOD and LOQ values are calculated from the linearity studies. Linearity study was performed three times and the value of slope and intercept were calculated from the calibration curve. The LOD was found to be 2.5 µg/ml and LOQ was found to be 7 µg/ml.

The percentage purity was calculated and it was found to be 99. The accuracy of the method was performed by recovery studies. To the pre analysed formulation a known quantity of Ritonavir raw material solution was added at different levels viz., 50%, 100%, 150%. The absorbances of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be 99.40-101.50%.. It was indicated that there was no interference with excipients used in the formulation during the analysis of Ritonavir. Hence the method was found to be accurate [13].

Precision studies were done with the Ritonavir standard (20 µg/ml). The absorbance of the Ritonavir standard at 243.5 nm was measured for six times and recorded. The % RSD value for six replicate absorbance was found to be 0.855% i.e., within the specified limit. Based on forced degradation studies according to the ICH requirements, this methodcan be used for the routine and quality control analysis of Ritonavir in bulk and pharmaceutical dosage forms.

Method development and Validation of Ritonavir by Visible Spectroscopy:

The recommended procedure is accurate, sensitive, and precise and is adapted to micro determinations of Ritonavir in bulk and pharmaceutical preparations. It may be used in routine determination of Ritonavir. The chemistry involved in the above proposed methods to give various colored chromogens can be explained by the following scheme. Ritonavir undergoes oxidation in the presence of ferric chloride and forms complex with MBTH reagent.

Table 3: Optical characteristics, regression data, precision and accuracy of the proposed methods for ritonavir

S.NO.	PARAMETER	LIMIT	Observation
1	λ_max (nm)	--	620
2	Beer's law limits (µg / ml)	--	2.5-12.5
3	LOD (µg / ml)	S/N ratio should be more than 3:1	0.140
4	Optimum photometric range (µg/ml)	--	2-15
5	Regression equation (y = mx+c)	--	Y = 0.049x + 0.128
6	Slope (b)	--	0.049
7	Intercept (c)	--	0.128
8	Correlation coefficient (R)	R^2 NLT 0.999	0.999
9	Specificity	no interfernces	specific
10	precision	%RSD NMT 2.0%	0.56
11	accuracy	range: 98-102%	99.10
12	LOq	S:N ratio should be more than 3:1	0.424
13	Robustness	%RSD NMT 2%	0.283 & 0.571

4. CONCLUSION

In the present investigation potent drug namely Ritonavir only few analytical methods were reported and hence there is wide scope for the development of new analytical methods for its quantitative analysis. All the developed methods are

complimentary to each other and the proposed methods can be used as alternative methods to reported ones thereby providing a wide choice for routine determination of the Ritonavir in bulk and pharmaceutical preparation.

1.) *Stability indicating RP-HPLC method:*

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ritonavir in bulk and pharmaceutical dosage forms.The results expressed in **Tables: 7-19** for HPLC is promising. In addition to positive requirements for analytical methods, the striking advantage of all the presently developed methods is that they are economical. And method is validated in terms of accuracy, precision, linearity, and limit of detection, limit of quantitation, and robustness. This method can be used for the routine determination of Ritonavir in bulk and pharmaceutical dosage forms.

2.) *Effect of solvents on max:*

Chromophores (e.g., Ritonavir) give rise to "characteristic" absorbance bands. Changes in theirenvironment (solvent) cause changes in their energy levels which then affect the wavelength and theintensity of absorbance. The experiment shows that external factors, in this case the solvent used, canalso influence the chromophore. Because the substance used shows both strong and weakabsorbance bands at different wavelengths, four different solvent concentrations will beexamined. This concludes that max changes based upon solvent for the same drug molecule and the Ritonavir showed different max in different solvents.

3.) *Titration :*

The proposed method of non-aqueous potentiometric titration was found to be precise, accurate and rugged. It requires simple apparatus as compared to methods reported in literature. The values of percentage recovery and standard deviation showed sensitivity. The method was completely validated. It showed satisfactory data for all the parameters of validation. Hence it can be applied for routine quality control application. Hence method is strongly recommended for quality control of method of Ritonavir.

4.) *UV spectroscopy:*

Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. Majority of the methods reported for Ritonavir estimation by UV Spectroscopy were by methanol which is costlier than 0.1N HCl. The present work demonstrates simple, rapid,accurate, reproducible and economical method for the estimation of Ritonavir by UV using 0.1N HCl. It is highly suitable for routine analysis of the drug to monitor quality control of drug products.

5.) *Visible spectroscopy:*

Among the several techniques [HPLC, GC, NMR, MS, IR] available for the assay of drugs, the visible spectrophotometric technique is simple and less expensive. The selectivity and sensitivity of the visible spectrophotometric method depends only on the nature of the chemical reactions involved in color development. A simple, sensitive and reproducible visible spectrophotometric method have been developed for the quantitative determination of Ritonavir in bulk drug and pharmaceutical dosage forms using MBTH and Ferric chloride by oxidation coupling.

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