Original Article

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

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Pharmaceutical analysis occupies a pivotal role in statutory 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 sample¹. Quantitative analysis is the determination of the absolute or relative amounts of
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 forms²,³.

2. MATERIALS AND METHOD

Drug profile⁴,⁵
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-y][methyl]]carbamoyl]amino]butanamido]-1,6-diphenylhexan-2-y]carbamate.
Molecular formula: C₃₇H₄₈N₆O₅S₂
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-HPLC⁶

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 Tablets⁷
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 up to 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 (160 g/ml) was injected into the chromatographic system, area for the Ritonavir peak was measured and the % Assay was calculated by using the formula⁸,⁹.

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 solutions¹⁰. Optical characteristics were given in table no.1

Table 1: Validation parameters of Ritonavir by HPLC

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>ACCEPTANCE CRITERIA</th>
<th>Ritonavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range</td>
<td>Correlation coefficient r² &gt; 0.999 or r² = 0.99982</td>
<td>8.0 – 240µg/ml</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>LOQ S/N &gt; 2 or 3</td>
<td>0.172064 µg/ml</td>
</tr>
<tr>
<td>LOD S/N &gt; 10</td>
<td>0.521407 µg/ml</td>
<td></td>
</tr>
<tr>
<td>System Precision</td>
<td>RSD &lt; 2%</td>
<td>RSD = 0.456</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>RSD &lt; 2%</td>
<td>RSD = 0.385</td>
</tr>
<tr>
<td>Method precision</td>
<td>RSD &lt; 2%</td>
<td>RSD = 0.298</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Recovery 98-102% (individual)</td>
<td>% recovery = 98.08-101.9</td>
</tr>
<tr>
<td>Specificity</td>
<td>1) No interference from blank, placebo and other degradation products with the main peak.</td>
<td>No interference.</td>
</tr>
<tr>
<td></td>
<td>2) Purity angle &lt; threshold angle</td>
<td>Peak pure</td>
</tr>
<tr>
<td>Solution Stability</td>
<td>&gt; 12 hour</td>
<td>Stable up to 24 hour %RSD = 0.55</td>
</tr>
<tr>
<td>Robustness</td>
<td>RSD NMT 2% in modified condition</td>
<td>Complies</td>
</tr>
<tr>
<td></td>
<td>Flow minus %RSD= 0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flow plus %RSD= 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic plus %RSD= 0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic minus %RSD= 0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wavelength plus %RSD= 0.094</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wavelength minus %RSD= 0.107</td>
<td></td>
</tr>
</tbody>
</table>
**Effect of solvents on \( \lambda_{max} \) 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. \( \lambda_{max} \) of Ritonavir in various solvents were tabulated.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>( \lambda_{max} ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>239</td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>240</td>
</tr>
<tr>
<td>3</td>
<td>0.1N HCl</td>
<td>241.5</td>
</tr>
<tr>
<td>4</td>
<td>0.1N NaOH</td>
<td>240.5</td>
</tr>
</tbody>
</table>

**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 the cost of Methanol, solubility of the drug was tried with 0.1NHCl and to increase the solubility initially 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 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 0.128.

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 measured at 243.5 nm. 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 method can be used for the routine and quality control analysis of Ritonavir in bulk and pharmaceutical dosage forms.
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 λ max:
Chromophores (e.g., Ritonavir) give rise to “characteristic” absorbance bands. Changes in their environment (solvent) cause changes in their energy levels which then affect the wavelength and the intensity of absorbance. The experiment shows that external factors, in this case the solvent used, can also influence the chromophore. Because the substance used shows both strong and weak absorbance bands at different wavelengths, four different solvent concentrations will be examined. This concludes that λ max changes based upon solvent for the same drug molecule and the Ritonavir showed different λ 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 the 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 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 determination 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.

5. REFERENCES


Conflict of Interest: None

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