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DEVELOPMENT AND VALIDATION OF COLORIMETRIC METHODS FOR THE DETERMINATION OF RITONAVIR IN TABLETS

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ABSTRACT

Two simple, precise and sensitive visible spectrophotometric methods (A and B) were developed for the quantitative estimation of ritonavir in bulk drug as well as in pharmaceutical dosage forms (tablets). Method A is based on the complex formation reaction of ritonavir with 1, 10-phenanthroline in presence of ferric chloride to form blood red colored chromogen with absorption maximum at 510 nm and Beer's law is obeyed in the concentration range of 20-100 μg/mL. Method B is based on the complex formation reaction of ritonavir with potassium ferricyanide in presence of ferric chloride to form bluish green colored chromogen with absorption maximum at 452 nm and Beer's law is obeyed in the concentration range of 20-100 μg/mL. The proposed methods are statistically validated and found to be useful for the routine determination of in tablets.

Key words: Ritonavir, Colorimetry, Tablets, Validation.

INTRODUCTION

Ritonavir is an inhibitor of HIV protease with activity against human deficiency virus-HIV[1]. Chemically, it is 10-hydroxy-20-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl-4-thiazolyll]-3, 6-dioxo-8, 11 -bis (phenyl methyl) -2, 4, 7, 12- tetraazatridecan-13-oic acid, 5- thiazoly methy lester, [5S*, 8R*, 10R*, 11R*)][2]. Literature review revealed that very few analytical methods including HPLC[3] methods for the analysis of ritonavir in pharmaceutical dosage forms are reported. Hence, it was thought worthwhile to develop spectrophotometric method for the same. In the present work, two simple and sensitive colorimetric methods (A and B) have been developed for the estimation of ritonavir in bulk drug and pharmaceutical dosage forms. Method A is based on the complex formation reaction of ritonavir with 1, 10-phenanthroline in presence of ferric chloride to form blood red colored chromogen with

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absorption maximum at 510 nm and Beer's law is obeyed in the concentration range of 20-100 μg/mL. Method B is based on the complex formation reaction of ritonavir with potassium ferricyanide in presence of ferric chloride to form bluish green colored chromogen with absorption maximum at 452 nm and Beer's law is obeyed in the concentration range of 20-100 μg/mL. The spectrophotometric parameters are established for standardization of the methods including statistical analysis of data.

EXPERIMENTAL

Instrumentation

All spectral and absorbance measurements were made on Shimadzu UV-VIS spectrophotometer - 1650.

Reagents

(i) 1, 10-Phenanthroline (0.3% in water)

(ii) Aqueous ferric chloride (0.5%w/v)

(iii) Potassium ferricyanide (0.2%w/v in water)

All reagents used were of analytical grade.

Preparation of standard solution

A 500 μg/mL ritonavir solution was prepared by dissolving 25 mg of drug in 50 mL of methanol.

Sample preparation

Twenty tablets were weighed and powdered. A quantity equivalent to 25 mg of ritonavir was weighed accurately, transferred to a beaker, dissolved in methanol, filtered through Whatmann filter paper No. 1 into 50 mL volumetric flask and made up to volume with methanol to get a concentration of 500 μg/mL.

Assay

Method A: Aliquots of ritonavir ranging from 1-5 mL were pipetted out into a series of 25 mL volumetric flasks. To each flask, 0.2 mL of ferric chloride and 1.5 mL of 1, 10-phenanthroline was added. It was heated for 30 min on the water bath at 95ºC and then cooled to room temperature. The volumes were made up to the mark with water. The

absorbance of the blood red colored chromogen was measured at 510 nm against reagent blank. The orange chromogen was stable for more than 4 hours. The amount of ritonavir present in the sample was computed from analytical curve, constructed by plotting concentration versus absorbance.

Method B: Aliquots of ritonavir ranging from 1-5 mL were pipetted out into a series of 25 mL volumetric flasks. To each flask, 1.0 mL of ferric chloride and 1.0 mL of potassium ferricyanide were added and set aside for 5 min. The volumes were made up to the mark with water. The absorbance of the bluish green colored chromogen was measured at 452 nm against reagent blank. The bluish green chromogen was stable for more than 4 hours. The amount of ritonavir present in the sample was computed from calibration curve.

Sample analysis

Pharmaceutical formulation of ritonavir was successfully analyzed by the proposed methods.

Appropriate aliquots were subjected to the above methods and the amount of ritonavir was determined from the calibration curves. The results of sample analysis are furnished in Table 2.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are furnished in Table 1. The regression characteristics like slope (b), intercept (a), correlation coefficient (r), percent relative standard deviation (% RSD) and standard error (SE) were calculated and the results are summarized in Table 1. The results of sample analysis showed that the drug determined by the proposed methods was in good agreement with the label claim proving the accuracy of the proposed methods.

Table 1: Optical characteristics and precision of the proposed methods A and B

Parameter	Method A	Method B
λ_{max} (nm)	510 nm	452 nm
Beer's law limits (μg/mL)	20-100	20-100
Molar absorptivity (L mol^{-1} cm^{-1})	8.083 x 10^3	9.903 x 10^3

Cont…

Parameter	Method A	Method B
Sandell's sensitivity (μg/cm^2 / 0.001 absorbance unit)	0.09001	0.07288
Regression equation (*y)	0.0105 x + 0.0243	0.01295 x + 0.0354
Slope (b)	0.01050	0.01295
Intercept (a)	0.0243	0.0354
Correlation coefficient (r)	0.99647	0.9989
% RSD	1.4697	0.7128
Standard error (SE)	0.0322	0.0214

*y = a + bc where c is the concentration of ritonavir in μg/mL

To study the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding a known amount of drug to preanalysed sample and the percentage recovery was calculated. The results are furnished in Table 2. The results indicate that there is no interference of other ingredients present in the formulations. Thus, the proposed methods are simple, sensitive, economical, accurate and reproducible and useful for the routine determination of ritonavir in bulk drug and its pharmaceutical dosage forms.

Table 2: Assay and recovery of ritonavir in dosage forms

Method	Labeled amount (mg)	Amount obtained(mg)*	Percentage recovery **
A	100	99.71	99.72%
B	100	99.68	99.70%

* Average of six determinations
** Average of three determinations

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