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SPECTROFLUORIMETRIC METHOD DEVELOPMENT AND VALIDATION OF RITONAVIR IN BULK DOSAGE FORM

Radhika M. kavar*, Jayashree V. Patil*, Vaishanvi G. Malthane, Vrushali V. Lambhade ,Rutuja R. Patil Pranjal G. Tupkar , Vaishanvi G. Magar, P .R. Gawandar, Dr.K.R. Biyani

Anuradha college of pharmacy, Anuradha nagar, sakegaon, road chikhali ,Dist – Buldhana (M.S) India 443201

Abstract :- A new, simple and cost-effective fluorescence spectrofluorimetric method was developed for the quantification of Ritonavir in bulk dosage forms. The method was established by measuring the fluorescence of Ritonavir in pH 4.7 acetate buffer at 372 nm after excitation at 485 nm. A linear response was observed in the concentration range of 10-50 µg/mL. This method is supported by analysis of various evidence cited in the ICH guidelines. The detection limit and value (0.7420002 and 3.710001 µg/mL, respectively) indicate that the method is accurate, precise and reproducible (% relative standard deviation <2.0). Therefore, the developed method is simple and can be effectively used for routine analysis of Ritonavir in pharmaceutical applications.

Keywords:- Ritonavir , Spectrofluorimetry, Accuracy, Linearity.

INTRODUCTION :- Ritonavir is a protease inhibitor drug used to treat HIV and AIDS. Ritonavir is often used with advanced anti-inflammatory drugs not because of its antiviral properties but because it inhibits the same enzymes that metabolize other antiviral drugs. This limitation results in higher plasma levels of the second drug, allowing doctors to reduce its dose and frequency and increase its therapeutic effect. Its structure is shown in the picture(fig:-1)

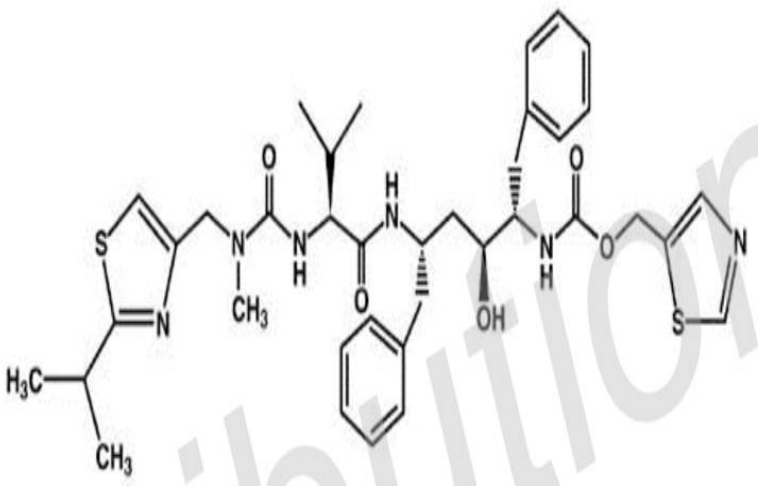

Fig 1:- Structure of Ritonavir

The chemical name of ritonavir is (5S, 8S, 10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11bis(phenylmethyl)-2, 4, 7, 12-Etraazatridecan-13-Oic Acid 5-thiazolyl methyl ester. It is official in Indian language Pharmacopoeia . and United States Pharmacopoeia[4]. From the literature search, it has been found that ritonavir can be measured by the spectrophotometric method, reversed-phase high-performance liquid chromatography (RP-HPLC), and other analytical skills methods. and HPTLC method . Apart from the methods mentioned above, spectrofluorimetric method was developed for the quantification of Ritonavir in bulk dosage forms for the quantitative determination of ritonavir in prescription drugs. The design is simple, clear, precise and clear. Statistical analysis has shown that this method is reproducible and selective for the analysis of ritonavir in drugs and tablets.

MATERIALS AND METHODS :-

Materials:-

Analytical pure ritonavir was received as a gift from a well-known pharmaceutical company. The solvents and chemicals used in this study are of analytical purity.

Instrumentation :-

Spectrofluorometer. The mean number (AM), standard deviation (SD), and relative percentage of standard deviation (%RSD) were calculated using statistical methods in MS-EXCEL.

Methods

Preparation of buffer

Acetate buffer of pH 4.7 was prepared by dissolving anhydrous sodium acetate (8.4 g) and glacial acetic acid (3.35 mL) in sufficient distilled water. The pH is then adjusted to 4.7 and the volume was made up with distilled water to produce 1000 ml .

Preparation of standard stock solution of Ritonavir

Ritonavir stock solution was produced by dissolving 8.75 mg of the analyte in ethanol (10 mL) by ultrasonication (1000 µg /mL).

This solution was appropriately diluted to get 10 µg/mL of Ritonavir in acetate buffer pH 4.7 and the same was utilized for finding optimum emission and excitation wavelengths.

Construction of calibration:

curve A series of solutions containing Ritonavir in the concentration of 1-5 µg/mL in acetate buffer pH 4.7 were produced by serial dilution of the initial stock solution. The fluorescence intensities of the resulting solutions were recorded at 372 nm after excitation at 485 nm. Relative fluorescence intensities and final concentrations (µg/mL) were plotted on Y- and X-axis, respectively to afford calibration curve.

Analytical method validation :-

The analytical method was confirmed using different validation parameters, such as linearity, limit of detection, limit of quantification, accuracy and precision as per ICH guidelines.

Linearity :- The method linearity was established by preparing a series of solutions containing 1-5 µg/mL concentrations of Ritonavir in acetate buffer pH 4.7. The fluorescence intensities at 485 nm were recorded for triplicate solutions at each concentration. The results were graphed as a calibration curve.

Limit of detection (LOD) and limit of quantification (LOQ):-

The limit of detection (LOD) and quantification (LOQ) of the method were determined using samples containing very low concentrations of Ritonavir as per ICH guidelines. The LOD and LOQ were calculated using 3.3*(standard deviation/slope) deviation/slope), respectively.

Precision

The repeatability or intra-day precision of the present method was set by estimating the corresponding response three times on the same day for three distinct concentrations of Ritonavir (1, 2 and 3 µg/mL). The intermediate or inter-day precision was determined by estimating selected concentrations (1, 2 and 3 µg/mL) response in triplicate on three different days over a week period. The results of both the studies were expressed as percentage relative standard deviation (%RSD).

RESULT :-

optimization of Analytical method

The solubility of the Ritonavir was initially studied in various organic solvents (methanol, ethanol, dimethyl formamide, dimethyl sulfoxide), acids (hydrochloric acid, glacial acetic acid), buffers (phosphate buffers, borate buffers and acetate buffers) and surfactants (CTAB, Sodium lauryl sulphate and Tween-60). Solubility3 of the analyte was observed only in ethanol. and acetate buffer pH 4.7. Hence the initial analyte solution was made with ethanol (10 µg/mL) and later acetate buffer pH 4.7 was used for dilution (100 and 10 µg/mL). The drug exhibited intense fluorescence at 372nm following excitation at 485 nm.

A linear calibration curve for the method was obtained in the concentration range 10-50 µg/mL for Ritonavir at 372 nm. The calibration curve was shown in fig 3.

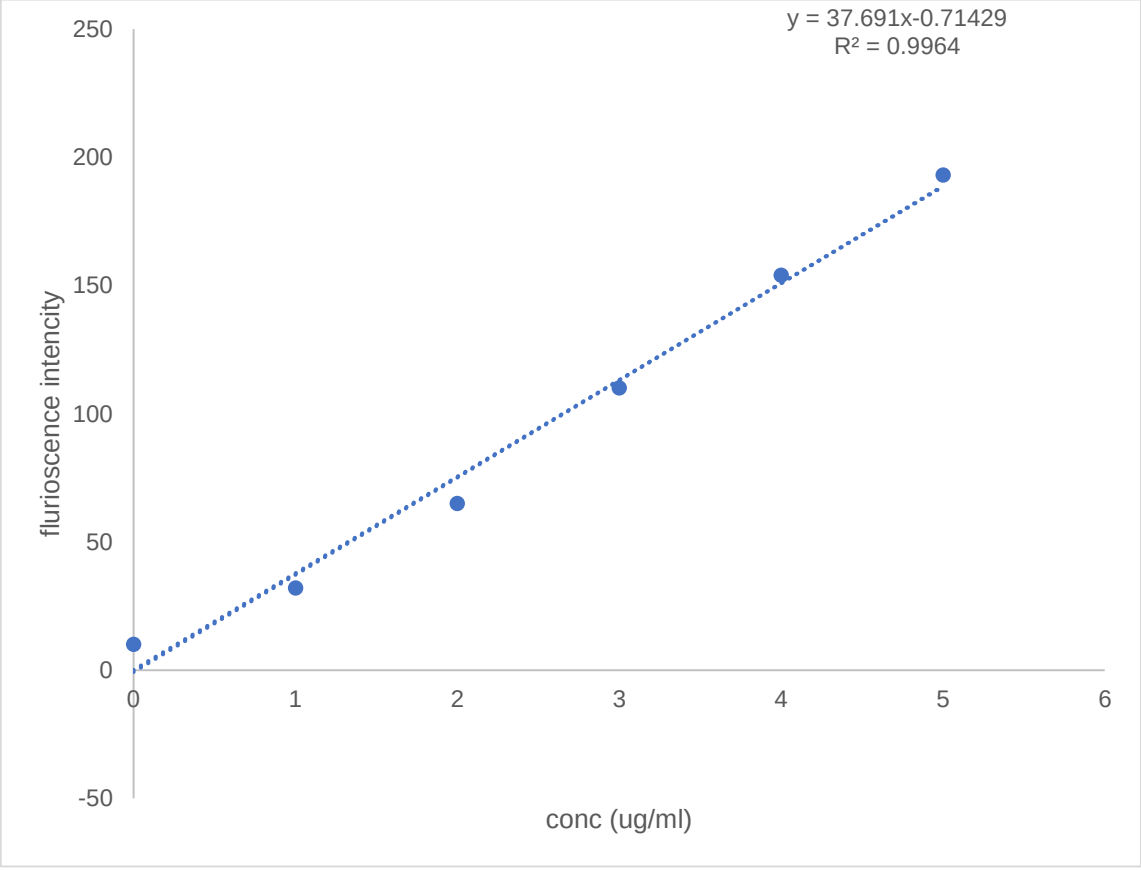

Figure 3 :- Calibration curve of Ritonavir

The results revealed a linear relationship between concentrations of Ritonavir and the relative fluorescence intensities. From the linear regression analysis correlation coefficient value (r2) of 0.9964 indicated to same.

Method validation :-

Linearity

The linearity was assessed by the least square regression method by measuring the responses of different concentrations of Ritonavir at emission wavelength 372 nm and the results were shown in Figure 1. The relationship between Ritonavir concentration and corresponding fluorescence intensity was found to be linear over the concentration range of 1-5 µg/mL with a r2 of 0.9964 The regression equation obtained was relative fluorescence intensity = 0.2147x -0.71429.

Limit of detection (LOD) and Limit of Quantification (LOQ) :-

Responsiveness of the method in terms of LOD and LOQ was determined by utilizing the formulae given in the experiment. The method resulted in LOD 0.7476385 , LOQ 3.7381925 µg/mL of LOD and LOQ, respectively. The summary of the system suitability parameters of the optimized method were given

Table 1: System suitability parameters of the method

Parameter	Values
Excitation wavelength (nm)	485
Emission wavelength (nm)	372
Linearity Range (µg/mL)	1 – 5
Correlation coefficient (r^2)	0.9964
Slope (m)	37.691
Intercept (c)	-0.71429
Standard deviation	67.77788
Regression equation	37.691x-0.71429
Limit of Detection (µg/mL)	0.7420002
Limit of Quantification	3.710001

Precision:-

Triplicate samples of three dissimilar concentrations containing 1, 2 and 3 µg/mL of Ritonavir were utilized for ascertaining the intra- and inter-day variability. Results of these studies were provided in Table 3. The %RSD values were found to be < 2.0, indicating good precision of the method.

Table 2: Precision data of the proposed method

Concentration (µg/mL)	Intra-day Amount found (AM ± SD) [a]	%RSD [b]	Inter-day Amount found (AM ± SD)[a]	%RSD b
1	66 ± 0.81	1.23	70 ± 1.24	1.76
2	71 ± 1.24	1.74	80 ± 0.81	1.02
3	85 ± 0.81	0.96	91 ± 0.81	0.89

AM: Arithmetic mean; SD: Standard deviation; RSD: Relative standard deviation; a Mean of three determinations; b Acceptance Criteria: %RSD < 2.0.

CONCLUSION :-

A simple and extraction-free spectrofluorimetric method was established for the quantification of Ritonavir using acetate buffer pH 4.7. When the method was validated as per ICH guidelines, it was proven to be accurate and precise. The content of Ritonavir in marketed tablets was estimated and the %RSD was found to be less than 2.0. Based on above results, we conclude that the developed spectrofluorimetric method could be routinely adopted in the quality testing of Ritonavir in pharmaceutical dosage forms.

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