Spectrofluorimetric quantification of ritonavir in bulk and pharmaceutical dosage form

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Abstract:A simple and sensitive spectrofluorimetric method has been developed for Ritonavir. Ritonavir exhibited good fluorometric intensities in solvents Methanol and 0.02 M SLS. This method was developed for estimation of Ritonavir at emission wavelength 349 nm, in 0.02 M SLS with linearity range of 0.2-3.2 µg/mL and good correlation coefficient of R2 value 0.9992. The limit of detection and limit of quantification for this method were 0.033 µg/mL and 0.10 µg/mL respectively. The developed method was statistically validated as per ICH guidelines. The %RSD value was found to be less than 2 for accuracy and precision studies. The results obtained were in good agreement with the labeled amounts of the marketed formulations. which was evidenced with %RSD value 0.009, The proposed analytical method is employed for routine quality control analysis of Ritonavir in tablet dosage forms.

Index Terms - Ritonavir, spectrofluorimetric, validation

I. INTRODUCTION

Ritonavir which is chemically 2 1,3-thiazol-5-ylmethylN-[(2S,3S,5S)-3-hydroxy-5- [(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-] methyl}) carbamoyl]amino}butanamido]-1,6-diph enylhexan-2-yl]carbamate is an orally administered drug used to treat HIV . It was originally developed as an inhibitor of HIV protease It is now rarely used for its own antiviral activity but remains widely used as a booster of other protease inhibitors. More specifically, ritonavir is used to inhibit a particular liver enzyme that normally metabolizes protease inhibitors, cytochrome P450-3A4(CYP3A4).

Extensive literature search revealed that various analytical methods have been reported for the determination of Ritonavir, such as spectrophotometric methods with different mobile phases and RP-HPLC[3] with different detectors and chromatographic conditions. In view of literature review, it was concluded that there is no reported spectrofluorimetric method for Ritonavir.

Since spectrofluorimetric is more sensitive and selective than absorption spectrophotometry, it has become a significant tool in drug analysis. Thus, the current work's goal was to create and verify a spectrofluorimetric technique that can be used to quantify ritonavir, taking into account studies on its solubility and degradation.

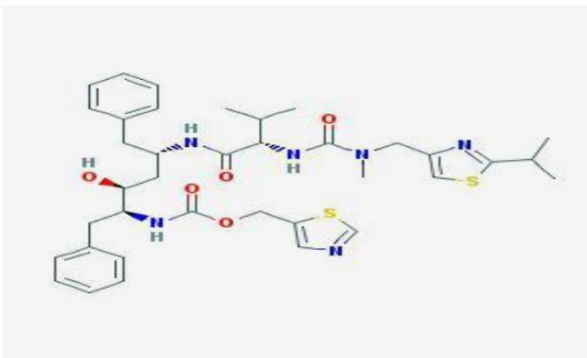

Fig.1 Chemical structure of Ritonavir

II. MATERIALS

Ritonavir standard drug was supplied by Hetero drugs Ltd, Hyderabad, India.

Methanol and sodium lauryl sulfate were supplied by SD fine – chem Ltd, Mumbai, India.

Instruments

A) Spectro fluorometer (Shimadzu model/RF 5301PC, Japan)
B) UV-Visible Spectrophotometer (1800, Shimadzu, Japan)
C) Ultra Sonicator (Sonica Ultrasonic Cleaner, Italy)
D) pH Meter (Elico L120, Hyderabad)
E) Digital Balance (Shimadzu AUX 220D, Japan)

III.METHOD DEVELOPMENT

Preparation of 0.02% sodium lauryl sulfate solution

Accurately weighed sodium lauryl sulfate (0.02gm) was transferred into 100 ml volumetric flask and volume is made up to the mark with 100 ml of distilled water.

Preparation of Ritonavir stock solution

Ritonavir (10mg) was weighed accurately and transferred into a 10ml volumetric flask and the volume is made up to the mark with Methanol(1000µg/ml). From this, 1ml solution was taken and diluted to 10 ml using 0.02% SLS to attain 100µg/ml. From this again 1ml solution was taken and diluted to 10ml with 0.02% SLS. The resulting 10µg/ml solution of Ritonavir was subjected for spectrofluorimetric method development.

Selection of analytical wavelengths for Ritonavir

Standard solution of Ritonavir was diluted appropriately with 0.02% SLS to obtain a final solution containing 10 µg/ml. Spectra of this diluted solution was scanned to get the excitation and emission wavelengths. The excitation wavelength was fixed and solutions were scanned to get the emission spectra.

Determination of λmax

The maximum absorption wavelength of Ritonavir was determined by using 10 µg/mL solution of ritonavir in 10 ml of methanol. Then, the standard solution was scanned using UV-visible spectroscopy at 200-400 nm and the UV[2] absorbance spectrum recorded and it was found to be 267 nm.

Calibration curve for Ritonavir

From the standard solution of Ritonavir (10 µg/ml), the serial dilutions of (0.2, 0.8, 1.4, 2.0 and 2.6, 3.2 mL from 10 µg/ml) were prepared by diluting with 0.02%SLS.The above dilutions gave final concentration of 0.2-3.2 µg/mL of Ritonavir The fluorescence intensities of the solutions were measured at the λmax.

Analytical validation

The developed method was validated[1] as per International Conference on Harmonization (ICH) guidelines to prove that the analytical method can be useful for quality control of the drug. [ICH guidelines (2003)]

Linearity studies

The standard concentrations of Ritonavir (0.2-3.2 µg/mL) were quantified, and fluorescence intensities were recorded and a calibration curve was developed.

Accuracy (recovery studies): Tablet powder equivalent to 10 mg of Ritonavir was transferred into three different 10 mL volumetric flasks and to it 80, 100 and 120% of standard drugs were spiked and made up the volume up to mark with 0.02% SLS.The amount of Ritonavir was estimated by measuring response at λmax 349 nm. The recovery was verified by estimation of drugs in triplicate preparations at each specified concentration level.

Precision

The intra-day precision was determined by estimating the corresponding response three times on the same day for three different concentrations of Ritonavir i.e., 0.2, 1.4 and 3.2 µg/ml.

The inter-day precision was determined by estimating the corresponding response three times on 3 different days over a period of 1 week for three different concentrations of Ritonavir (0.2, 1.4 and 3.2 µg/mL).

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) for the procedure were performed on samples containing very low concentrations of analyte Ritonavir under the ICH guidelines by following formulae.

$LOD = 3.3\ \sigma / S$ $LOQ = 10\ \sigma / S$

σ = Standard deviation of the response

S = Slope of the calibration curve of the analyte

Assay of ritonavir in pharmaceutical dosage form

Twenty tablets containing 100 mg of Ritonavir were taken and accurately weighed.An accurately weighed quantity of powder equivalent to 10 mg (amount of powder taken 67.4 mg) was transferred to 10 ml volumetric flask and volume was made up to the mark with methanol. The above stock solution was filtered through Whatman filter paper (No.41). From the filtrate 1.0 mL was transferred into a 10 mL volumetric flask and the volume was made up to the mark with 0.02%SLS to give a solution containing 100 µg/mL Ritonavir. Further 1.0 mL of the above solution is transferred into a 10 mL volumetric flask to give a standard of 10 µg/ml. This solution was used for the estimation Ritonavir. The amount of Ritonavir was determined by substituting responses into the equation of the straight line representing the calibration curves, with correction for dilution.

IV. RESULTS AND DISCUSSION

Determination of λmax

The standard solution of Ritonavir 10 µg/mL was scanned between 400 to 200 nm Methanol as a solvent. The UV absorbance spectrum of Ritonavir was shown in the following Figure:2 From the spectra, the λmax of Ritonavir was found to be 267 nm, which is in accordance with the literature (λmax =260 nm).

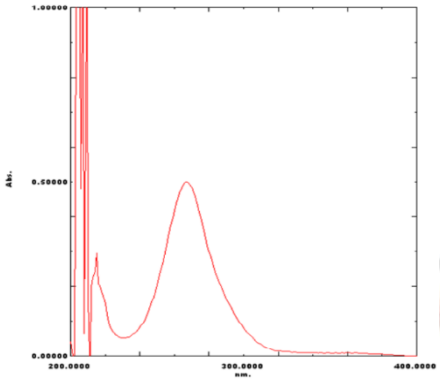

Figure 2: UV spectrum of ritonavir (10 µg/mL) in methanol.

Method optimization (Optimization of concentration of Ritonavir)

The optimum concentration of SLS was determined by preparing 10 µg/mL solution of Ritonavir in different concentrations of SLS (0.1, 0.3, 0.5 and 0.7%). The solutions were scanned for fluorescence. The results are provided in Table 4.4. Hence the least concentration 0.2 i.e., 0.02 M SLS was considered as optimum for the analysis.

Fluorescent spectrum for Ritonavir

Stock solution of Ritonavir dissolved in Methanol was diluted appropriately with 0.02 M SLS to obtain the final standard solution containing 10 µg/mL. Spectrum of this diluted solution was scanned to get the excitation and emission wavelengths. The excitation wavelength was fixed, and solutions were scanned to get the emission spectra. Ritonavir exhibited inveterate fluorescence of at 1.856 emission wavelength at 349 nm after excitation at 270 nm, in 0.02 M SLS (Figure 3) λmax 270 nm

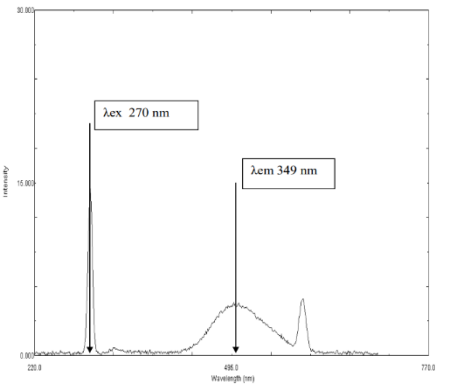

Figure 3: Fluorescent spectrum of Ritonavir (10 µg/mL) in 0.02 M SLS at excitationwavelength of 270 nm and emission wavelength 349nm

Calibration curve for Ritonavir

The calibration curve for Ritonavir at 349 nm was plotted (figure 5) and it revealed that Ritonavir was showing linear relationship over the concentration range of 0.2-3.2 µg/mL. From the linear regression analysis correlation coefficient value (R^2) for Ritonavir was found to be 0.9992, which indicates the linearity of the method.

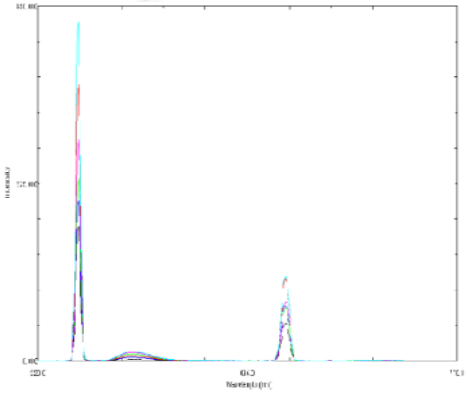

Figure 4 : Emission overlay spectra for Ritonavir

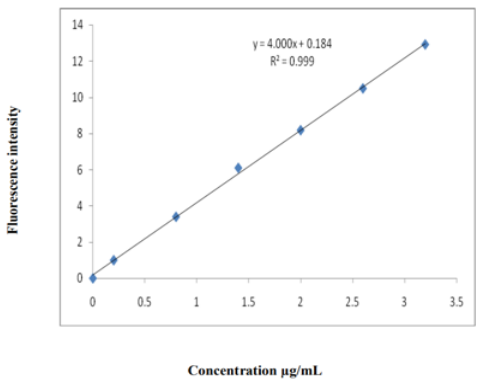

Figure 5: Calibration plot of Ritonavir

The linearity was evaluated by the least square regression method. The responses for Ritonavir at λmax 349 nm were found to be linear in the concentration range of 0.2-3.2 µg/mL,with a correlation coefficient (R 2) value of 0.999. The regression analysis of the calibration curves was shown in Figure no.4,and it was observed that with the increase in Ritonavir concentration, the response at λmax 349 nm was increased.

Accuracy (recovery studies)

The mean of percentage recoveries and %RSD values was calculated and reported in Table 1

The % recoveries of Ritonavir for three levels are found to be satisfactory.

Analyte	**Recovery level%**	**Conc of sample (µg/mL)**	**Conc of standard spiked (µg/mL)**	**Total amount (µg/mL)**	**Amount recovery (AM±SD) (µg/mL) (n=3)**	**%Recovery**	**%RSD**
Ritonavir	80%	1.0	0.8	1.8	1.852±0.02	107.2%	0.18
	100%	1.0	1.0	2.0	2.012±0.01	111.5%	0.49
	120%	1.0	1.2	2.2	2.505±0.04	116.3%	1.59

Table 1: Data for accuracy studies of Ritonavir

Precision

In both intra-day and inter-day precision studies, the %RSD of repeatability was found to be within acceptance criteria (less than 2.0). Thus, the results indicated good precision of the developed method.

Concentration (µg/mL)	Intra-day precision		Intra-day precision	
	Concentration estimated(µg/mL) (AM ± SD) (n=3)	%RSD	Concentration estimated(µg/mL) (AM ± SD) (n=3)	%RSD
0.2 µg/mL	0.97±0.01	1.02	0. ±0.01	1.12
1.4 µg/mL	1.13±0.02	1.76	1.43±0.02	1.39
3.2 µg/mL	3.12±0.03	0.96	3.13±0.03	0.95

Table 2: Data for precision of the analytical method

Limit of detection (LOD) and Limit of quantitation (LOQ)

From the linearity plot the LOD and LOQ of Ritonavir was calculated, which indicates the sensitivity of the method.

Assay of ritonavir in pharmaceutical dosage form

The accuracy of the proposed method was evaluated by the assay of commercially available tablets (NORVIR) containing Ritonavir (100 mg)[7]. The results obtained for Ritonavir was compared with the corresponding labelled amounts. The amount of Ritonavir in the formulation (NORVIR) was found to be 100.06 ± 0.10 mg. This amount was within the limits. The % assay in commercial formulations was found to be 100.06, which is in the acceptance range of 80-120% as per ICH guidelines. The %RSD for formulation (NORVIR) was less than 2, which indicates the accuracy of the proposed method.

Optimized chromatographic conditions.

The details of optimized conditions for spectrofluorimetric method of Ritonavir were given in Table 3

S. No.	Parameter	Ritonavir
1	Excitation wavelength (nm)	270 nm
2	Emission wavelength (nm)	349 nm
3	Linearity range (µg/mL)	0.2 - 3.2 µg/mL
4	Slope (m)	4.000
5	Intercept (c)	0.184
6	Regression equation	Y =4.000+0.184
7	Correlation coefficient (R^2)	0.9992
8	Accuracy (%RSD)	< 2
9	Precision ((%RSD)	< 2
1 0	LOD (µg/mL)	0.033 µg/mL
11	LOQ (µg/mL)	0.10 µg/mL
12	Assay (%)	100.06 %

Table 3: optimized conditions

V.CONCLUSION

The present work was attempted with the objective to develop and validate a simple spectrofluorimetric method for Ritonavir. The conclusions drawn from the above work are mentioned below. Ritonavir exhibited good fluorometric intensities in solvents Methanol and 0.02 M SLS.Simple spectrofluorimetric method was developed for estimation of Ritonavir at emission wavelength 349 nm, in 0.02 M SLS with linearity in the range of 0.2-3.2 µg/mL with R2 value 0. 9992.The contemplated analytical method was validated as per ICH guidelines. The % relative standard deviation (%RSD) found to be less than 2, denotes the immense scope of sensitivity, precision, accuracy and system suitability of the analytical method. LOD and LOQ values obtained were 0.033 µg/mL and 0.10 µg/mL, respectively.

Ritonavir content was estimated by the contemplated analytical method in marketed tablets. The % assay of Ritonavir was found to be 100.06. The assay values were in good agreement with labeled claims, which was evidenced with %RSD value 0.009, denoting interference of excipients in the drug estimations was not found.

Based on the above results, we concluded that the proposed analytical method is employed for routine quality control analysis of Ritonavir in tablet dosage forms.

VI. REFERENCES

[1] Chiranjeevi, K. and Channabasavaraj, K.P., 2011. Development and validation of RP-HPLC method for quantitative estimation of ritonavir in bulk and pharmaceutical dosage forms. *International Journal of Pharmaceutical Sciences and Research*, *2*(3), p.596.

[2] Hemanth Kumar, A.K., Sudha, V., Leelavathi, A. and Ramachandran, G., 2016. A rapid isocratic high performance liquid chromatography (HPLC-UV) method for the quantification of ritonavir in human plasma. *International Journal of Pharmacology and Pharmaceutical Sciences*, *8*(7), pp.64-68.

[3] Gowthami, K., Fatima, G., Farheen, M., Yasmeen, F., Afreen, S., Sailaja, M., Saidabi, S. and Moqeemoddi, S., 2012. Sensitive analytical method development and validation of ritonavir bulk drugs by RP-HPLC. *Journal Of Scientific Research In Pharmacy*, *1*(1), pp.20-22.

[4] Müller, A.C. and Kanfer, I., 2010. An efficient HPLC method for the quantitative determination of atazanavir in human plasma suitable for bioequivalence and pharmacokinetic studies in healthy human subjects. *Journal of pharmaceutical and biomedical analysis*, *53*(1), pp.113-118.

[5] Peruri, V.V.S. and Musuluri, M., 2011. A RP-HPLC Method for the Estimation of Ritonavir in Pharmaceutical dosage forms. *Journal of Pharmacy Research*, *4*(9), pp.3049-3051.

[5] Benjamin, T. and Ramachandran, D., 2015. Development and validation of RP-LC method for Dapsone in pharmaceutical formulations. *World J Pharm Res*, *20*, pp.1090-1099.

[6] Gandhi, S.V. and Rasika, R.K., 2016. A RP-HPLC method development and validation for the estimation of ritonavir in bulk and pharmaceutical dosage form. *Journal of Chemical and Pharmaceutical Research*, *8*(7), pp.901-904.

[7] Trivedi, C.D., Mardia, R.B., Suhagia, B.N. and Chauhan, S.P., 2013. Development and validation of spectrophotometric method for the estimation of ritonavir in tablet dosage form. *International Journal of Pharmaceutical Sciences and Research*, *4*(12), p.4567..

[8] Skoog, D.A. and West, D.M., 1963. Fundamentals of analytical chemistry. *(No Title)*..

[9] Salunke, J.M., Pawar, D.S., Chavhan, V.D. and Ghante, M.R., 2013. Simultaneous UV spectrophotometric method for estimation of ritonavir and lopinavir in bulk and tablet dosage form. *Der Pharmacia Lettre*, *5*(3), pp.156-162.

[10] Peruri, V.V.S. and Musuluri, M., 2011. A RP-HPLC Method for the Estimation of Ritonavir in Pharmaceutical dosage forms. *Journal of Pharmacy Research*, *4*(9), pp.3049-3051.

[11] Seetaramaiah, K., Smith, A.A., Ramyateja, K., Alagumanivasagam, G. and Manavalan, R., 2012. Spectrophotometric determination of ritonavir in bulk and pharmaceutical formulation. *Scientific Reviews & Chemical Communications*, *2*, pp.1-6.

[12] Hemanth Kumar, A.K., Sudha, V., Leelavathi, A. and Ramachandran, G., 2016. A rapid isocratic high performance liquid chromatography (HPLC-UV) method for the quantification of ritonavir in human plasma. *International Journal of Pharmacology and Pharmaceutical Sciences*, *8*(7), pp.64-68..

[13].Shilpa, Z., Dyaneshwar, P., Shubhangi, W., Sagar, J., Ganesh, J. and Shital, W., 2011. Analytical Uv Spectroscopic Method Development and Validation for the Estimation of Ritonavir. *World Journal of Pharmacy and Pharmaceutical Sciences*, *2*(6), pp.5473-5490.