The Pharma Innovation Journal 2019; 8(4): 30-34

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 30-34

www.thepharmajournal.com
Received: 16-02-2019
Accepted: 20-03-2019

Mohammad Yasir Peerzade
MCE Society Allana College of Pharmacy Azam Campus Pune, Maharashtra, India

Shakeel Memon
MCE Society Allana College of Pharmacy Azam Campus Pune, Maharashtra, India

Dr. Kiran Bhise
M.C.E, Society Allana college of Pharmacy,Azam campus, Pune., Maharashtra, India

Ansari Irfan Aamer
University Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India

Correspondence
Mohammad Yasir Peerzade
M Pharm (Pharmaceutics)
MCE Society Allana College of Pharmacy, K.B, Hidayatullah Road Azam Campus Pune, Maharashtra, India

Development and validation of UV-Visible spectrophotometric method for estimation of ritonavir in bulk and formulation

Mohammad Yasir Peerzade, Shakeel Memon, Dr. Kiran Bhise and Ansari Irfan Aamer

Abstract
Aim: To develop and validate a simple, precise and cost-effective UV- visible spectrophotometric method for the estimation of Ritonavir according to the ICH Q2 (R1) guideline.
Methods: Spiked Ritonavir solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of Ritonavir were prepared, and absorbance was recorded at wavelength of maximum absorbance. Calibration curve of concentration vs. absorbance was plotted and linearity and range was calculated. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, robustness and ruggedness were calculated using QC standards. In order to check the performance, pre-validated method was used to estimate the Ritonavir content of marketed formulation.
Results: The wavelength of maximum absorbance for Ritonavir was found to be 255 nm. The correlation coefficient over the concentration range of 30-210 µg/mL was found to be 0.999. Developed UV method was found to be precise during the intra-day and inter-day study and showed percent relative standard deviation in the range of 0.21 to 1.60 & 0.21 to 1.62 respectively. The total percent recovery of Ritonavir was found to be 99.49 to 100.39 %. Developed method was found to be robust and rugged for the intended use. Developed UV-Visible method was successfully used for the estimation of Ritonavir content in marketed formulation of reputed Indian pharmaceutical industry.
Conclusion: A simple, precise and cost-effective UV- visible spectrometry method for the estimation of Ritonavir was developed. The said method was developed using economical percentage of organic phase in aqueous media as solvent. Said validated UV- visible method can be efficiently used for the estimation of Ritonavir in bulk as well as formulation.

Keywords: UV- visible spectrometry, ritonavir, validation

Introduction
Ritonavir is (5S, 8S, 10S, 11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3, 6-dioxo-8, 11-bis (phenyl methyl)-2, 4, 7, 12-etraazatridecan-13-oic acid 5-thiazolyl methyl ester. (Fig. 1). It is official in Indian Pharmacopoeia [1-2] and United States Pharmacopoeia [3-4]. Ritonavir is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Ritonavir is frequently prescribed with Highly Active Anti-Retroviral Therapy, not for its antiretroviral action, but as it inhibits the same host enzyme that metabolizes other protease inhibitors. This inhibition leads to higher plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy [5-6].

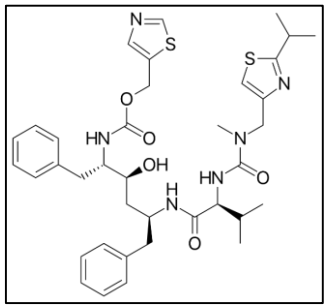

Fig 1: Chemical structure of Ritonavir

In spite of therapeutic vitality and extensive utility of Ritonavir, there are limited scientific reports that demonstrate the development and validation of analytical methods for its estimation in bulk and formulation. Therefore, considering the therapeutic importance of the Ritonavir and the need of simple yet precise and robust analytical method for the same, it was envisaged that development of UV-Visible spectrophotometric method for the determination of Ritonavir in bulk and the formulation by using co-solvent system consisting economic percentage of organic solvent will be worth.

Experimental

Instruments and reagents

A double beam UV-visible spectrometer (UV-730, Jasco) with spectra manager software were used for the analysis. Quartz cells having 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Mettler Toledo) with internal calibration mode was used for the accurate weighing purpose. Ritonavir was obtained as gift sample from Lupin Pharma Ltd, Aurangabad. Methanol was purchase from Merck. All the chemicals of analytical grade were used for the proposed study.

Preparation of working standard drug solution

The standard Ritonavir (5mg) was accurately weighed and transferred into the (5ml) Volumetric Flask and dissolved properly and diluted up to the mark with the mixture of Water: Methanol (40:60 v/v) to achieve a final concentration of 1000 µg/mL (Stock-1). Further Stock-1 was properly diluted using mobile phase to achieve a 100 µg/mL (Stock-2) solution.

Determination of wavelength of maximum absorbance (λmax)

The Stock-2 was scanned utilizing full output mode with medium scanning speed for a whole range of UV/VIS Spectrophotometer, the ranging from 800-200 nm with a co-solvent system as a blank. After acquiring the spectrum, λmax was identified. The above method was repeated thrice.

Preparation of calibration curve

The Calibration curve was prepared by using Stock-2 to accomplish the seven-diverse calibration standard representing 30, 60, 90, 120, 150, 180, 210 µg/mL strength. An absorbance of every calibration standard was estimated at λmax 255nm using fixed wavelength measurement mode. The calibration curves representing concentration vs. absorbance was plotted utilizing in Microsoft Excel 2016. Previously mentioned technique was rehashed multiple time with the goal that reproducible outcomes can be obtained

Method Validation

Developed UV method for the estimation of Ritonavir was validated in terms of parameters like linearity, range, precision, robustness, ruggedness, accuracy, limit of quantification (LOQ) and limit of detection (LOD) using predefined calibration standards as portrayed below [9-10].

Linearity and range

Linearity of the proposed UV method was established using seven different calibration standards. Based on analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis.

R square value was considered to be important factor for establishing linearity of the proposed method. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy

The accuracy of the proposed UV method was evaluated using recovery studies after standard addition of analyte of interest. Three different solutions of Ritonavir were prepared in triplicate at level of 80%, 100% and 120% of its predefined concentration (30, 120, 210 µg/mL). In predefined concentrations, different amount of ritonavir was included (standard addition method) and accuracy was determined based on percent recovery. For calculating the percent recovery, following equation was utilized

%RC= (SPS-S/SP) × 100

Where,
% RC = Percent recovery
SPS = Amount found in the spiked sample
SP = Amount added to the sample
S = Amount found in the sample

Intra-day precision and Inter-day precision

Precision of the assay method was assessed in terms of repeatability by carrying out seven independent assays of ritonavir test arrangement and the % RSD of measurement (intra-day). Intermediate precision of the method was checked by performing same methodology on three consecutive days.

Robustness

Robustness the developed UV method was obtained utilizing different percentage of methanol in a co-solvent system. Methanol percentage in a co-solvent system was purposefully changed 45 and 55%. Ritonavir (90 µg/mL) was prepared utilizing above mentioned co-solvent system independently, (n=5) and sample was analyzed at λmax 255 nm for Ritonavir content. The result was determined in terms of % RSD.

Ruggedness

Ruggedness, the UV/VIS method was carried out by analyzing triplicate sample of Ritonavir utilizing two distinct instruments (V-730, Jasco and V-630, Jasco). The result was depicted in terms of % RSD.

Limit of Quantification (LOQ)

In UV method development LOQ was determined by utilizing the following equation.

LOQ = 10xSD/S

Where, S= slope
SD= Standard deviation of Y-intercepts

Limit of Detection (LOD)

In UV method development LOD was determined by utilizing the following equation

LOD =3.3×SD/S

Where, SD= Standard deviation of Y-intercepts
S= Slope

Estimation of Ritonavir content in marketed formulation

Developed & prevalidated UV-Vis method was successfully used for estimation of Ritonavir content in marketed

formulation. For the study, Ritomune Tablets were purchased from local market of Hyderabad and contents of Tablet were collected and suitable dilution were made using pre-optimized co-solvent system. Prepared samples were analyzed using pre-validated UV-method & results were reported in terms of average percent assay.

Results and Discussion

Method development and optimization

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for Ritonavir solution (100 µg/mL) was carried out using full scan mode of UV-Visible spectrophotometer (Figure 2). Full scan was processed using UV software and the λmax was identified with the help of software. It was found to be 255 nm for Ritonavir

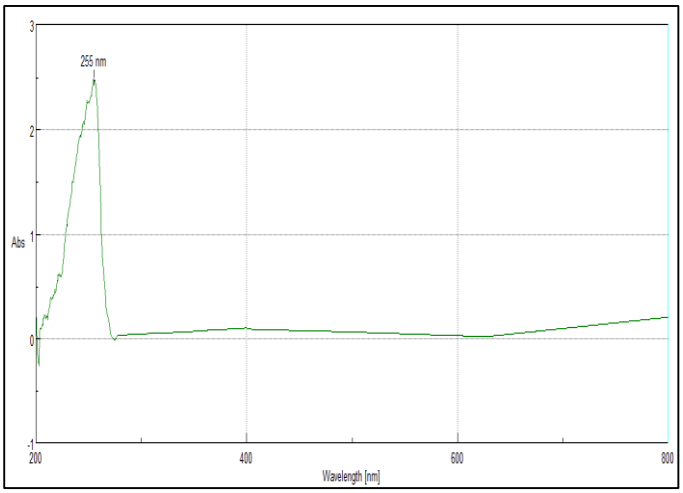

Fig. 2: UV-visible spectra of Ritonavir

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating correlation between concentration and the response. As compare to graphical metho0d, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of Ritonavir, calibration curve for Ritonavir was developed using seven different calibration standards. The absorbance of different calibration standards at 255 nm was recorded using fixed wavelength mode of UV-Visible spectrophotometer. Calibration curve was repeated three times and reported as shown in Table 1.

Table 1: Results of calibration curve at 255 nm

Standard	Conc (µg/ml)	Absorbance ±
CAL STD-1	30	0.0634 ±0.0020
CAL STD-2	60	0.1318±0.0015
CAL STD-3	90	0.1987 ±0.0038
CAL STD-4	120	0.2649 ±0.0040
CAL STD-5	150	0.3251 ±0.0017
CAL STD-6	180	0.3981 ±0.0055
CAL STD-7	210	0.4598±0.0038

Method Validation

Linearity and Range

Proposed method is to be used for its optimum performance. Considering the importance of linearity and the range, seven-point calibration curve of Ritonavir covering a range of 30-210 µg/ml was developed. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; y = 0.0022x + 0.0009 with correlation coefficient 0.999 as shown in Figure 3. From the linearity study, it was revealed that, developed UV method was linear in the pre-defined concentration range of calibration standards.

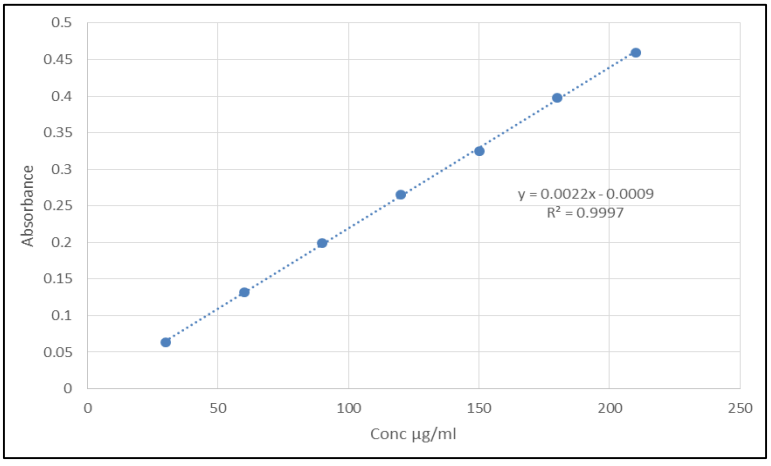

Fig 3: Calibration curve for Ritonavir

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of UV method for Ritonavir, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of Ritonavir was found to be 99.87% whereas at 100 and 120 % standard addition, it was found to be 100.39 and 99.49% respectively. % RSD was found to be less than 2 for the Ritonavir recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 99.49 to 100.39% and the % RSD was well below 2%.

Table 2: Accuracy data of UV method for Ritonavir

Sr. No.	Concentration (%)	Original level (µg/mL)	Amount added (µg/mL)	% Recovery	Mean % Recovery	% RSD
1	80	30	24	99.18	99.87	1.66
2	80	30	24	98.65		
3	80	30	24	101.77		
4	100	120	120	99.97	100.39	0.41
5	100	120	120	100.40		
6	100	120	120	100.81		
7	120	210	252	100.30	99.49	1.05
8	120	210	252	98.86		
9	120	210	252	98.29		

Precision

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at 30, 120 and 210 µg/ml levels of Ritonavir. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively. % RSD values of intra-day precision study were found to be in between 0.21 and 1.62 whereas those of inter-day precision study were in between 0.21 and 1.60. Overall, % RSD values of less than 2 showed the precision of developed UV method.

Table 3: Intra-day precision data of UV method for Ritonavir

S No.	Conc. (µg/mL)	Morning			Afternoon			Evening		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	30	0.0647	98.12	1.62	0.0646	97.90	1.33	0.0640	97.03	1.09
2	120	0.2647	100.27	0.21	0.2645	100.21	0.31	0.2652	100.48	1.26
3	210	0.4601	99.60	0.27	0.4604	99.65	0.25	0.4618	99.97	0.91

Table 4: Inter-day precision data of UV method for Ritonavir

S No.	Conc. (µg/mL)	Day 1			Day 2			Day 3		
		Mean	% Assay	%RSD	Mean	% Assay	%RSD	Mean	% Assay	%RSD
1	30	0.0644	97.68	1.07	0.0642	97.41	1.44	0.0640	97.28	1.60
2	120	0.2648	100.32	0.49	0.2641	100.05	0.38	0.2643	100.12	0.50
3	210	0.4608	99.74	0.21	0.4600	99.58	0.25	0.4605	99.62	0.29

Robustness

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is an important parameter of analytical method as a small, un-intentional change in method parameters like solvent composition; pH etc. may occur during routine use and may hamper the performance of said method. It is expected that such change should not alter the performance of the analytical method. Therefore, robust analytical method is preferred. Robustness of proposed UV method was established by modifying the composition of co-solvent system. Change in water and methanol percentage (55 to 65 %) in co-solvent system did not affect the method performance. % RSD values were found to be in between 0.32 and 0.38 as shown in Table 5. % RSD values below 2 showed that proposed UV method is robust in nature.

Table 5: Robustness data of UV method for Ritonavir

Sr. No.	Concentration (µg/mL)	% Methanol	Absorbance	% RSD
1	90	55	0.1987	0.38
2	90	65	0.2003	0.32

Ruggedness

Ruggedness of analytical method is the ability of a method to resist the change in its performance in spite of influential environmental factors like temperature. Rugged analytical methods are preferred as these methods are free from impact of environmental factors. In order to establish the ruggedness of proposed UV method, Ritonavir solution was analyzed at three different temperature conditions. Sample analysis and data processing resulted into % RSD values between 0.27 and 0.38. Results revealed that proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 6.

Table 6: Ruggedness data of UV method for Ritonavir

S. No.	Concentration (µg/mL)	Instrument Model & Make	Absorbance	% RSD
1	90	V-730, Jasco	0.1985	0.38
2	90	V-630, Jasco	0.1946	0.27

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was found to be 0.495 and 1.502 µg/ml respectively as shown in Table 7. Lower LOQ value indicated that proposed method would be suitable for analyzing the samples containing even small quantities of Ritonavir.

Table 7: LOD & LOQ data for UV method for Ritonavir

1	LOD	0.495 µg/mL
2	LOQ	1.502 µg/mL

Estimation of Ritonavir

The developed UV method was successfully applied for the estimation of Ritonavir content in Ritomune Tablet IP 100 mg. Average percent assay of Ritonavir tablet was found to be 99.98 %.

Conclusion

A simple, accurate and precise UV-Visible spectrophotometric method for the estimation of Ritonavir was developed and validated. The Proposed method was found to be robust and rugged in nature and was successfully used for the estimation of Ritonavir.

References

1. Indian Pharmacopoeia. 2007; III:1058
2. United States Pharmacopoeia, National Formulary. 2007; 25:2143.
3. Kasper, Braun W, Fauci, Hauser, Longo, Jameson. Harrison's Principles of Internal Medicine, 16th ed, USA, 2004, 1132.
4. Skoog, Holler, Nieman. Principles of Instrumental Analysis, fifth edition, Thomson Asia Pvt. Ltd., Singapore, 2004, 300-325.
5. Beckett A, H Stenlake. Practical Pharmaceutical Chemistry, 4th edition, CBS Publishers and Distributors, New Delhi, 2002; 2:275-295.
6. Jyoti M, Dnyaneshwar S, Vinit Chavhan D, Minal R. Simultaneous UV spectrophotometric method for estimation of ritonavir and lopinavir in bulk and tablet dosage form, Der Pharmacia Lettre. 2013; 5(3):156-162.
7. Shilpa Z, Dyaneshwar P, Shubhangi W, Sagar J, Ganesh J, Shital W. Analytical Uv Spectroscopic Method Development and Validation for the Estimation of Ritonavir, World Journal of Pharmacy and Pharmaceutical Sciences. 2011; 2(6):5473-5490.
8. Veera VS, Murali M. A RP-HPLC Method for the Estimation of Ritonavir in Pharmaceutical dosage forms, Journal of Pharmacy Research. 2011: 4(9):3049-3051.
9. Note for guidance on validation of analytical procedures: text and methodology. European Medicines Agency, 1995, 1-15.
10. Validation of analytical procedures: text and methodology Q2 (R1). ICH harmonized tripartite guideline, 1994.