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Research Article

Development and Validation of an Innovative Stability Indicating Method Using UV-Spectroscopy Techniques for Ritonavir in Bulk Drug and Pharmaceutical Dosage Forms

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Abstract: Ritonavir is a protease inhibitor used to treat HIV/AIDS. It is seldom employed for its antiviral activity but instead as a booster for other protease inhibitors. Our study's significant objective is to develop a new, simple, accurate, precise, and reproducible UV spectrophotometric approach for investigation by apotheosis to analyze ritonavir. Three alternative simple, accurate, and precise UV spectrophotometric techniques-the, zero-order (method A), first-order (method B), and the area under the curve (method C) spectrophotometric methods have been established for the measurement of ritonavir in bulk and pharmaceutical dosage. The drug was dissolved in ethanol, and then 0.063 M Phosphate buffer solution (pH 7.0), and the observed λ_{max} are 271 nm for the zero-order spectrophotometric method (method A), 258 nm for the first-order spectrophotometric method, 260–281 nm for the area under the curve spectrophotometric method (method C). Under the optimum conditions, linear relationships with good correlation coefficients 0.9994–0.9999 were found between the reading and the corresponding concentration of the drug in the range of 10-50 µg/ml. The proposed methods can detect the analyte in the lower limits of 0.24 to 0.38 µg/ml. The precision of the methods was satisfactory, and the percentage relative standard deviation values did not exceed 2%. The proposed methods were successfully applied to the analysis of ritonavir in its bulk and commercial formulations with good repeatability and reproducibility; the label claim percentages ranged from 99.56 to 99.64 ± (0.34-0.63) % w/v. The research findings of the current approach were shown to be more accurate and trustworthy for ritonavir in pharmaceutical dosage forms and bulk pharmaceuticals compared to those previously reported by the spectrophotometric approaches. The proposed techniques can be used for routine inspections without causing any interference from excipients or other substances because of the quick and repeatable analysis.

Keywords: Ritonavir, Zero order spectroscopy, Derivative spectroscopy, First order spectroscopy, Area under the curve spectroscopy, Ritomune, ICH guidelines.

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1. INTRODUCTION

The analysis is vital in any product or service and is also important in drugs because it involves life. The number of drugs introduced in the market is increasing every year. These drugs may be either new entities or partial structural modifications of the existing ones. Thus, the development and validation of analytical methods play an essential role in drug discovery, development, and manufacturing; hence, developing newer analytical methods for such drugs is mandatory[1]. Method development is the process that proves that the analytical method is acceptable for use. Various novel analytical techniques like UV-spectrophotometric, RP-HPLC, LC-MS, and LC-MS-MS are useful for the standard Control and Quality Assurance of Pharmaceuticals and, therefore, the safety of patients[2]. The analytical method's validation provides information on several stages and parameters, including accuracy, precision, linearity, detection limit, quantification, specificity, range, and robustness. Validation should be carried out in compliance with legal requirements like the ICH standards[3]. Even though spectrophotometric methods are frequently preferred, particularly by small-scale industries, because the equipment costs less and the maintenance problems are minimal, UV-spectrophotometric techniques are quite simple, accurate, precise, reproducible, and sensitive among all techniques. The analysis approach is based on evaluating the monochromatic light's absorption by colorless substances in the near ultraviolet path of the spectrum (200-380 nm)[4]. Thus, in the present research, apotheosis developed a new, simple, sensitive, precise, reproducible UV spectrophotometric method to determine ritonavir in bulk drug and pharmaceutical dosage forms. Human immunodeficiency virus (HIV) is one of the most overwhelming diseases affecting a large pediatric and adult population worldwide. Ritonavir is a protease inhibitor used to treat HIV/AIDS[5]. Ritonavir is one of the potent synthetic HIV protease inhibitors, approved by the US Food and Drug Administration (FDA) between 1995 and 1997, that have revolutionized HIV therapy[6]. Ritonavir (Figure 1) is (5S, 8S, 10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3, 6-dioxo-8, 11-bis (phenylmethyl)-2, 4, 7, 12-tetraazatridecan-13-oic acid 5-thiazolyl methyl ester[7]. Ritonavir can also inhibit severe acute respiratory syndrome (SARS) or novel coronavirus[8]. Ritonavir is also an inducer of several metabolizing enzymes [CYP1A4, glucuronosyl transferase (GT), and possibly CYP2C9 and CYP2C19]; the magnitude of drug interactions is difficult to predict, particularly for drugs that are metabolized by multiple enzymes or have low intrinsic clearance by CYP3A[9]. Ritonavir is highly protein-bound with a half-life of 3–5 hours and will decrease its metabolism due to the auto-induction of the CYP3A4 isoenzyme system[10]. In contrast, the known adverse effects include diarrhea, drowsiness, heartburn, change in the ability to taste food, and headache[11].

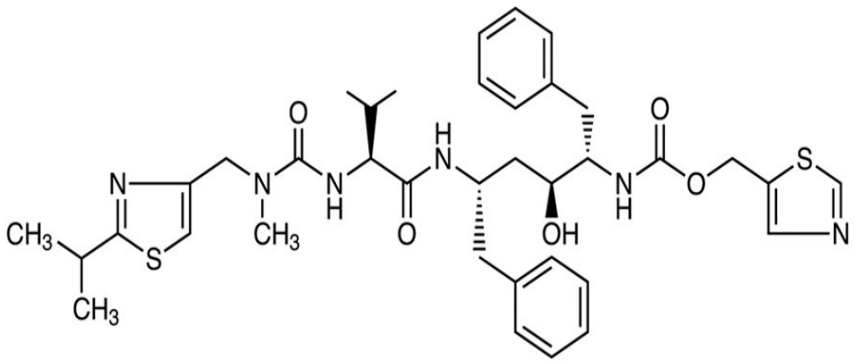


Fig 1: Chemical structure of ritonavir

Ritonavir quantification has been reported using various reported analytical techniques and tools; reported crucial UV spectroscopic approaches are the development and validation of the spectrophotometric method for the estimation of lopinavir and ritonavir in tablet dosage form[12], New sensitive UV- spectrophotometric method for simultaneous estimation of lopinavir and ritonavir in fixed-dose combination as soft gels[13], Development, and validation of various UV spectrophotometric methods for the estimation of famciclovir in bulk and its formulation[14], A new second-derivative spectrophotometric method for the determination of vildagliptin in pharmaceutical dosage form[15]. HPLC techniques by determining the maximum absorbance are the evaluation of an international pharmacopeia method for the analysis of ritonavir by liquid chromatography[16], Determination of saquinavir and ritonavir in human plasma by RP-HPLC and the analytical error function[17], A simple HPLC method for simultaneous determination of lopinavir, ritonavir, and efavirenz[18], The high-performance liquid chromatographic determination of ritonavir in human plasma, cerebrospinal fluid, and saliva[19], LC determination of ritonavir, an HIV protease inhibitor, in soft gelatine capsules[20], Simultaneous high-performance liquid chromatographic determination of the antiretroviral agents' amprenavir, nelfinavir, ritonavir saquinavir, delavirdine, and efavirenz in human plasma[21], Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography[22], Quantitative estimation of lopinavir and ritonavir in tablets by RP-HPLC method[23], RP-HPLC method development and validation for simultaneous estimation of lopinavir and ritonavir in dosage form and in plasma[24], and the reported method for determine ritonavir by chromatographic separation with mass detection is Rapid and sensitive method for the quantitative determination of lopinavir and ritonavir in human plasma by liquid chromatography-tandem mass spectrometry[25]. Based on the previously mentioned analytical techniques, our primary aim and object are to develop efficient, rapid, sensitive, selective, linear, and accurate UV-spectroscopy approaches for determining ritonavir and performing stability-indicating stress degradation studies. The procedure was assessed based on ICH and USP standards[26,27]. According to ICH Q2R1 recommendations, the drug concentration of ritonavir in various pharmaceutical products is assessed using linearity, accuracy, precision, specificity, the limit of detection (LOD), and quantification (LOQ)[28].

2. MATERIALS AND METHODS

2.1 Materials

Mylan Laboratories Pvt. Ltd., India, provided standard ritonavir bulk powder as a kind gift. Ritonavir was purchased using the local pharmacy's branded tablet ritomune (100 mg). The investigation used only chemical reagents of analytical quality. Analytical grade ethanol was procured from GlaxoSmithKline Pharmaceuticals Limited in Mumbai, India. From Gujarat, India's Ideal Chemicals Pvt. Ltd., we received dipotassium hydrogen phosphate, sodium hydroxide, hydrogen peroxide, and hydrochloric acid.

2.2 Instrumentation

Shimadzu 1800 UV spectrophotometer was used for this analysis, with 1 cm matched quartz cells for all measurements and UV probe 4.2 series software. The investigation employed a digital analytical balance (Mettler Toledo, India), an ultrasonic sonicator (Spectra Lab, India), and validated borosilicate glass pipettes, volumetric flasks, and beakers.

2.3 Preparation of 0.063 M Phosphate Buffer Solution (pH 7.0)

Dissolve 5.18 g of anhydrous disodium hydrogen phosphate and 3.65 g of sodium dihydrogen phosphate monohydrate in 950 ml of distilled water and adjust the pH with phosphoric acid; then adjust the remaining volume of 1000 ml volumetric flask with distilled water.

2.4 Preparation of Standard Stock Solutions for UV-spectroscopy

Ritonavir was accurately weighed at 10 mg and then transferred to a volumetric flask with a 10 ml capacity. The solute was first dissolved in the ethanol to make a solution to make the standard stock solution with a concentration of 1,000 µg/ml. This solution was then further sonicated and diluted with the 0.063M Phosphate buffer solution (pH 7.0) to the desired level. To achieve the working standard with a 100 µg/ml concentration, further dilute the previously prepared standard stock solution with the 0.063M Phosphate buffer solution (pH 7.0). The solutions with the requisite concentrations for procedures A, B, and C were diluted with the 0.063M Phosphate buffer solution (pH 7.0) made from the working standard.

2.5 Different Methods of Development

A stability indicator test method can be defined as a "validated quantitative analytical method capable of detecting the change over time in the chemical, physical or microbiological properties of the pharmaceutical substance and specific pharmaceutical products so that the content of active ingredients and degradation products can be accurately measured without interference[29]. Developing the method proves that the application is suited for an analytical approach. Thus, in the present investigation, ascendance created an innovative UV spectrophotometric approach to analyse ritonavir in bulk, and pharmaceutical dosage form, three different easy, accurate, and precise UV spectrophotometric techniques zero-order (method A), first-order (method B), and area under the curve (method C) have been developed, followed by the ICH guidelines[27] and Mondal *et al*., (2020)[30].

2.6 Method A (Zero order spectrophotometric method)

The UV-Spectroscopy principle is used to conduct numerous analyses in the simplest possible manner. The 0.063M Phosphate buffer solution (pH 7.0) was kept as a blank sample. Samples from 200 to 400 nm were taken. The linearity investigation revealed that the maximum wavelength (λ_{max}) is 271 nm.

2.7 Method B (First-order spectrophotometric method)

The UV-Spectroscopy principle is used to conduct numerous analyses in the simplest possible manner. The 0.063M Phosphate buffer solution (pH 7.0) was kept as a blank sample. Spectra between 200 and 400 nm were measured. The zero-order spectra were transformed into first-order derivative spectra (delta lambda 8, scaling factor 1) using the inbuilt software of the instrument. After interpreting the data for linearity, the λ_{max} was 258 nm.

2.8 Method C (Area under the curve spectrophotometric method)

Two effective areas on the mixed spectrum directly proportional to the concentration of the desired spectral component effectively solve the broad spectrum with the methodology. A reference solution was preserved for the 0.063M Phosphate buffer solution (pH 7.0). Samples were captured between 200 and 400 nm. Using UV probe software 2.42, the spectra between 260 to 281 nm were recorded. The area versus concentration data was used to conduct the linearity assessment.

2.9 Method Validation

The developed method was validated according to the ICH guidelines (ICH Q2R1) for linearity, specificity, precision, accuracy, robustness, the limit of detection, and quantification[26,27].

2.10 Linearity

Linearity is an analytical technique that achieves test results proportionate to the analyte concentration in the test sample. A plethora of solutions was made for the standard calibration curve based on Breer's Lambert law for methods A, B, and C at 10-50 µg/ml.

2.11 Precision

The analytical method, or precision, denotes the reproducibility of the analytical process. Precision is the degree of agreement between individual test results when a technique is subjected to numerous samplings of a homogenous sample. Six concentrations of 30 µg/ml (methods A, B, and C) of standard drug solution are evaluated for intraday and interday precision, and variations are investigated. The drug concentrations were evaluated on different consecutive days in the intermediate precision investigation, demonstrating the laboratory variation on different days. The percentage RSD was calculated.

2.12 Accuracy

The analytical technique of accuracy examines the degree to which test findings and the actual value are near one another. Accuracy was assessed at three distinct concentration levels (50%, 100%, and 150%) by appropriately incorporating ritonavir standard stock solution into the sample. The amount of drug in triplicate preparations at each concentration level and the percent recovery were used to calculate the recovery.

2.13 Robustness

The effect of small, deliberate changes in the optimized method was studied by robustness evaluation. To evaluate the robustness of the developed method, the parameter was deliberately varied[31]. However, deliberate changes to the analytical process's parameters demonstrate its consistency over time. It was performed by altering the UV-spectrophotometric technique's wavelength (±2 nm). Still, there was no apparent difference in the results within ICH guidelines. A sample evaluation was done six times.

2.14 Sensitivity

The quantification and detection limit were used as parameters in the sensitivity calculation. LOD refers to the lowest analyte concentration in a sample that can be detected but not fundamentally quantified. The lowest level at which an analyte may be measured with acceptable accuracy and precision is known as the LOQ. The following formula was used to calculate LOD and LOQ.

$$LOD = 3.3 \times standard\ deviation\ of\ response/slope\ of\ the\ calibration\ curve$$
$$LOQ = 10 \times standard\ deviation\ of\ response/slope\ of\ the\ calibration\ curve$$

2.15 Analysis of Commercial Dosage Form

UV-vis spectroscopy imaging is a non-intrusive and simple-to-operate analytical technique that holds the potential to provide a mechanical foundation for formulation development. The application of UV imaging over the past five years demonstrated its potential use in various drug substances and delivery systems. It is also qualitatively and quantitatively useful in some more specialized research. This technique is frequently used in many other industries as a kinetic and monitoring study to ensure authenticity analysis, quality monitoring, and purity[32]. Our research also illustrates the assay of the commercial dosage form of 100 mg ritomune tablets of ritonavir. To analyze various commercial tablet dosage forms based on the efficacy and pharmacokinetics study evaluation and carry out the assay to determine the amount of drug present in the dosage forms. The 100 mg brand-name ritomune tablets of ritonavir (20 tablets) were compared in this dissertation. For evaluation, 20 tablets are accurately energized and weighed. The powdered tablet containing the equivalent of 10 mg of ritonavir was weighed and placed into a volumetric flask with a capacity of 10 ml. The ethanol was added to the mark and sonicated, and all solutions were filtered. Measure tablets by contrasting them to the reference standard drug.

2.16 Stress Degradation Studies

Since it impacts the drug product's safety and effectiveness, the chemical stability of pharmaceutical compounds is a major source of concern. The FDA and ICH guidelines state the requirement of stability testing data to understand how a drug's substance and product quality change with time under various environmental factors. Knowledge of the molecule's stability helps select the proper formulation and package and provide proper storage conditions and shelf life, which is essential for regulatory documentation. So, we also emphasize stress degradation studies as specified in regulatory guidelines [33].

2.17 Oxidation Stress Degradation Studies

The 1 ml of ritonavir stock solution was combined with 1 ml of 3% hydrogen peroxide, diluted with ethanol up to 10 ml, and left at room temperature for 90 minutes. The reference solution underwent the same conditions without adding 3% hydrogen peroxide. The test solution was sufficiently diluted to provide test solutions with concentrations of 30 µg/ml for Methods A, B, and C. At last, the samples were analyzed using UV spectroscopy to calculate the degradation percentage.

2.18 Acid Stress Degradation Studies

The 1ml of ritonavir stock solution was mixed with 1ml of 1N hydrochloric acid, and the volume was filled off with ethanol to 10 ml and maintained at room temperature for 90 minutes. The same conditions were applied to the reference solution without adding acid. The test solution was neutralized with NaOH and diluted adequately to get a test solution of 30 µg/ml for methods A, B, and C. The samples were also scanned in UV spectroscopy, and the degradation percentage was calculated.

2.19 Alkali Stress Degradation Studies

In addition, 1 ml of 1N NaOH was added to 1 ml of ritonavir stock solution. The volume was then filled to 10 ml with ethanol and left at room temperature for 90 minutes. Additionally, the reference solution was treated under identical circumstances without adding NaOH. Further, the solution was diluted to provide test solutions with 30 µg/ml concentrations for methods A, B, and C. To calculate the percentage of deterioration, the samples were further scanned using UV spectroscopy.

2.20 Dry Heat Stress Degradation Studies

The standard drug solution was kept in an oven at 80°C for 48 hours to assess dry heat degradation. Developed the 30 µg/ml test solutions for methods A, B, and C. The reference solution underwent the same procedures without the sample being heated. The sample deterioration percentages were also estimated after UV spectroscopy scanning the samples.

2.21 Photolytic Stress Degradation Studies

The sample solution was exposed to UV light at 365 nm for 48 hours in a UV chamber to test the drug's photolytic stability. Developed the 30 µg/ml test solutions for methods A, B, and C. The reference solution was also subjected to the same circumstances but without exposure to UV light. The degree of deterioration was also recorded after the samples were scanned using UV.

3. RESULTS

3.1 Linearity

In linearity studies, calibration curves were graphed in a 10-50 µg/ml concentration range for methods A, B, and C. The linear regression equation of method A is y = 0.0121x + 0.2238 with a correlation coefficient of 0.9994 (Figures 2 and 3), Method B is y = 0.0005x + 0.0087 with a correlation coefficient of 0.9989 (Figures 4 and 5), Method C is y = 0.0479x + 0.7817 with a correlation coefficient of 0.9999 (Figures 6 and 7).

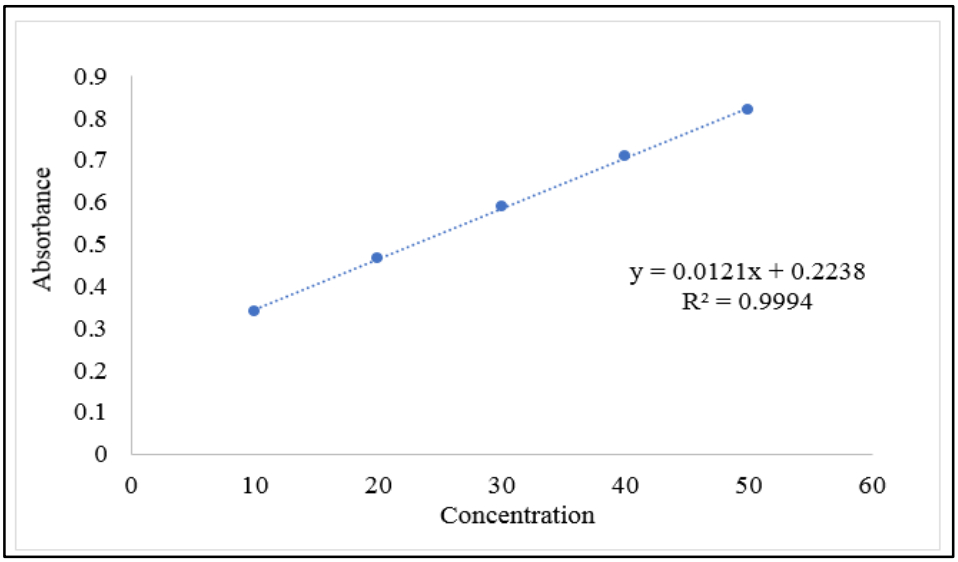


Fig 2: Calibration curve of ritonavir for method A (Zero order spectrophotometric method)

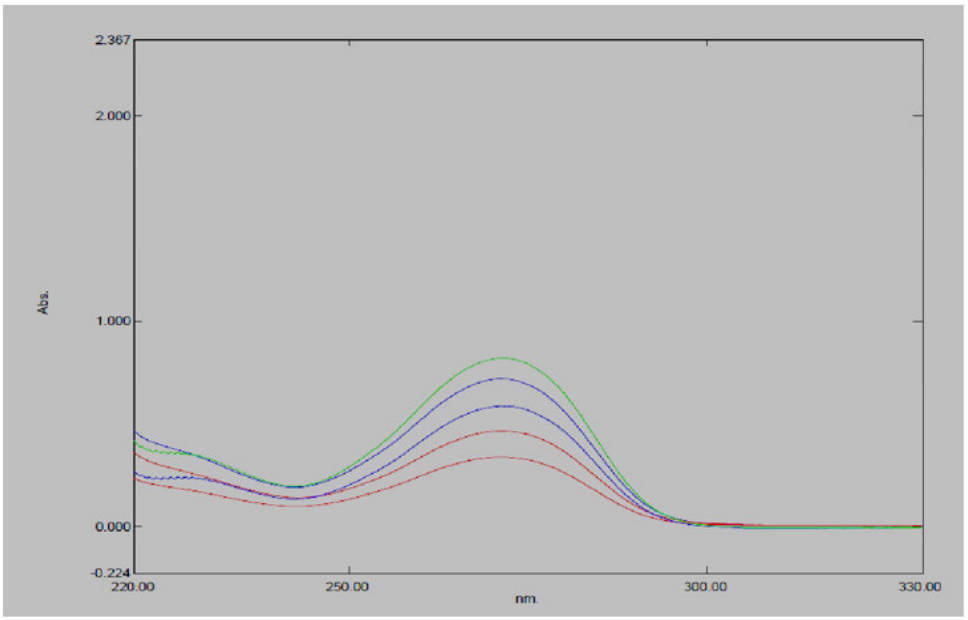


Fig 3: Overlay spectrum of ritonavir for method A (Zero order spectrophotometric method)

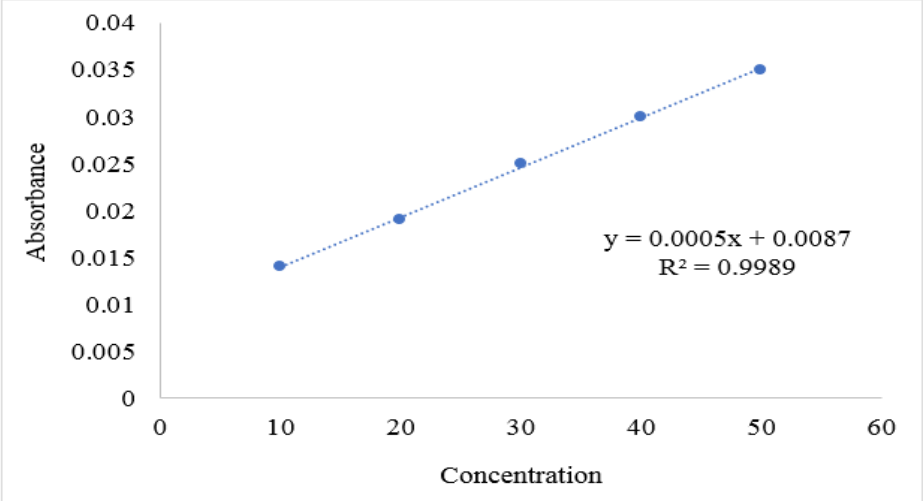


Fig 4: Calibration curve of ritonavir for method B (First-order spectrophotometric method)

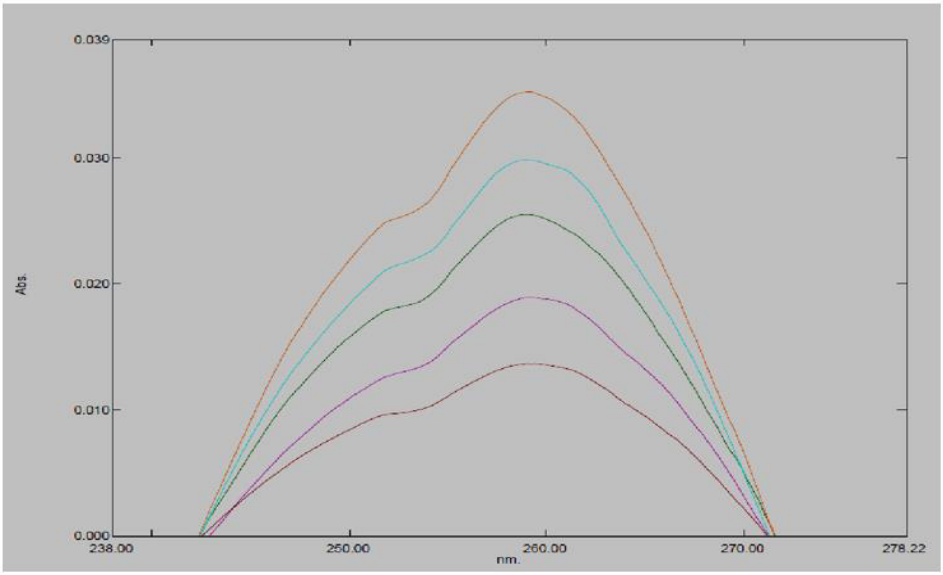


Fig 5: Overlay spectrum of ritonavir for method B (First-order spectrophotometric method)

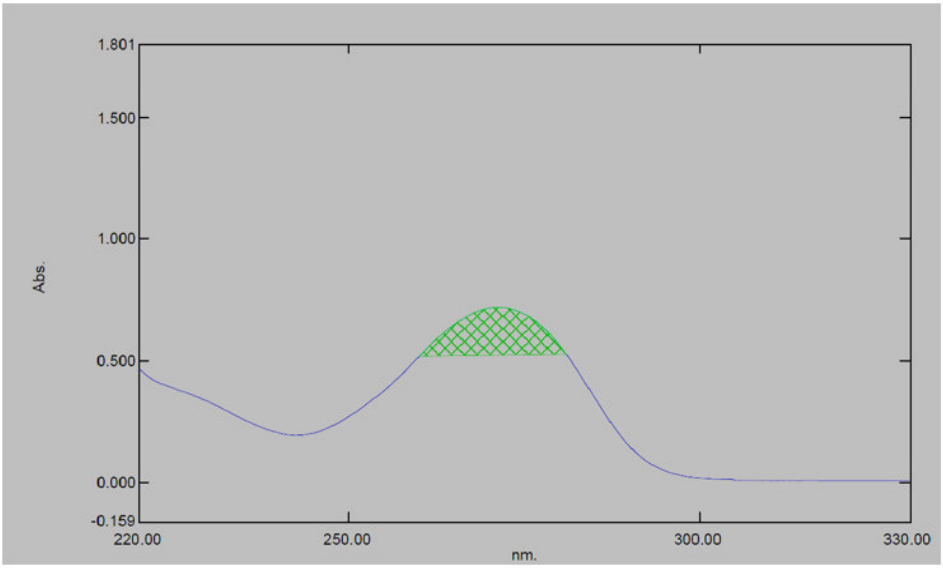


Fig 7: The spectrum of ritonavir for method C (Area under the curve spectrophotometric method)

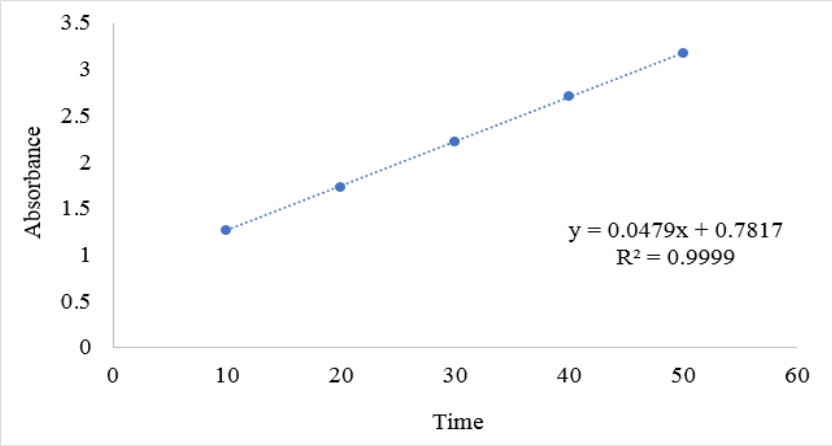


Fig 6: Calibration curve of ritonavir for method C (Area under the curve spectrophotometric method)

The following spectrum and calibration curves for methods A, B, and C represent the values of linearity, and the R^2 value is more than 0.99, which is accepted as per the ICH guidelines.

3.2 Precision

The performance of intraday and interday precision and the percent RSD for the response of six replicate measurements in methods A, B, and C were calculated. Results from the intraday and interday precision studies are summarized in Tables 1 and 2.

Table 1: Intraday precision for ritonavir methods A, B, and C

Sl.No.	Conc. (µg/ml)	Method A	Method B	Method C	%RSD		
		Absorbance		Area	Method		
					A	B	C
1	30	0.586	0.025	2.234	0.20 %	0 %	0.29%
2	30	0.587	0.025	2.216			
3	30	0.589	0.025	2.218			
4	30	0.589	0.025	2.219			
5	30	0.588	0.025	2.220			
6	30	0.587	0.026	2.218			

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), and Method C (Area under the curve spectrophotometric method).

Table: 2. Interday precision for methods A, B, and C of ritonavir

Day	Conc. (µg/ml)	Method A	Method B	Method C
		Absorbance ± S.D, (%RSD)		Area ± S.D, (%RSD)
1	30	0.564 ± 0.007, (1.24 %)	0.025 ± 0.00, (0 %)	2.218± 0.010, (0.45 %)
2	30	0.559 ± 0.003, (0.53 %)	0.025 ± 0.00, (0 %)	2.225± 0.013, (0.58 %)
3	30	0.670 ± 0.011, (1.64 %)	0.025 ± 0.00, (0 %)	2.235± 0.003, (0.13 %)

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), Method C (Area under the curve spectrophotometric method). The precision study's results are satisfactory. Because the percentage RSD value was less than 2%, as per the ICH criteria, these can be used for future investigations of other parameters.

3.3 Accuracy

The measurements were carried out to perform the accuracy study at three different levels of concentration 50, 100, and 150 % for methods A, B, and C. Table 3 represents the data observed in the accuracy study.

Table 3: Ritonavir accuracy observations for methods A, B, and C

Level	Conc. (µg/ml)	Amount of drug added (µg/ml)		Amount recovered (µg/ml)			% RSD		
		Pure	Formulation	Method			Method		
				A	B	C	A	B	C
50%	12.5	10	2.5	12.46	12.38	12.46	0.40 %	0.68 %	0.08 %
	12.5	10	2.5	12.52	12.35	12.47			
	12.5	10	2.5	12.42	12.51	12.45			
100%	25	10	15	24.89	24.89	24.87	0.20 %	0.04 %	1.01 %
	25	10	15	24.98	24.88	24.92			

	25	10	15	24.98	24.87	24.89			
150%	37.5	10	27.5	37.43	37.49	37.45	0.12 %	0.70 %	0.13 %
	37.5	10	27.5	37.34	36.98	37.48			
	37.5	10	27.5	37.40	37.34	37.38			

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), and Method C (Area under the curve spectrophotometric method).

The number of recovery values in the accuracy studies demonstrates that the proposed method is accurate and that the interference response does not exist. The percentage % RSD value was significantly good per the ICH guidelines and acceptable for the method development and carrying out the method validation study.

3.4 Robustness

The study of robustness was performed to change the wavelength of the proposed methods and calculate the percentage of RSD. Table 4 represents the data of the robustness study.

Table 4: The ritonavir robustness data for several approach techniques using UV techniques

Method	Condition	%RSD
A	Wavelength 269 nm	0.43
	Wavelength 273 nm	0.76
B	Wavelength 256 nm	1.27
	Wavelength 260 nm	1.24
C	Wavelength 258 nm to 279nm	1.36
	Wavelength 262 nm to 283 nm	1.03

Mean of six observations.
Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), and Method C (Area under the curve spectrophotometric method).

All the parameters were passed with no notable changes. The percent RSD was within the acceptable range, so the proposed method applies for the intended method development and allows the validation of other parameters study.

3.5 Assay

The commercially available ritomune (100 mg) formulations of ritonavir assay were carried out, and the purity percentage was assessed by methods A, B, and C. The interpretation findings for the marketed tablets of ritonavir are depicted in Table 5.

Table 5: Assay data for the commercially available ritonavir formulations (ritomune 100mg) using UV techniques

Drug and label claim	An amount estimated (mg/tab)			Purity (% w/w) ± S.D, (%RSD)		
	Method			Method		
	A	B	C	A	B	C
Ritomune 100mg	99 ± 0.67	99 ± 0.32	99 ± 0.23	99.56 ± 0.34 (0.34%)	99.61 ± 0.45 (0.23%)	99.64 ± 0.63 (0.42%)

Mean of three observations.
Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), and Method C (Area under the curve spectrophotometric method).

The assay results were compared with the standard drug, and no significant variance was observed. The amount of drug estimation was good, and the purity percentage was more than 99.00. The values are repeatedly indicating the significance of the proposed methods.

3.6 Sensitivity

The sensitivity of the proposed method was carried out as per the ICH guidelines. The limit of detection and quantification was calculated, and the obtained values indicated the detection and quantification limits of the prosed methods A, B, and C.

Table 6: Employing UV techniques, ritonavir sensitivity assessments (LOD and LOQ)

Method	LOD (µg/ml)	LOQ (µg/ml)
Method A	0.24	0.75
Method B	0.45	1.42
Method C	0.38	1.14

Mean of three observations.
Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), and Method C (Area under the curve spectrophotometric method).

In the LOD analysis, the detection limits for methods A, B, and C were 0.24, 0.45, and 0.38 µg/ml, while the quantitation limits were 0.75, 1.42, and 1.14µg/ml. Table 6 displays the relevant LOD and LOQ values for ritonavir.

3.7 Stress Degradation Studies

Studies on stress degradation were carried out under various stressful conditions, but no significant degradation was observed. The highest degradation percentage was observed in oxidation stress tribunals, where methods A, B, and C observed 17.29, 20.00, and 20.04% of degradation, respectively (Figure 8).

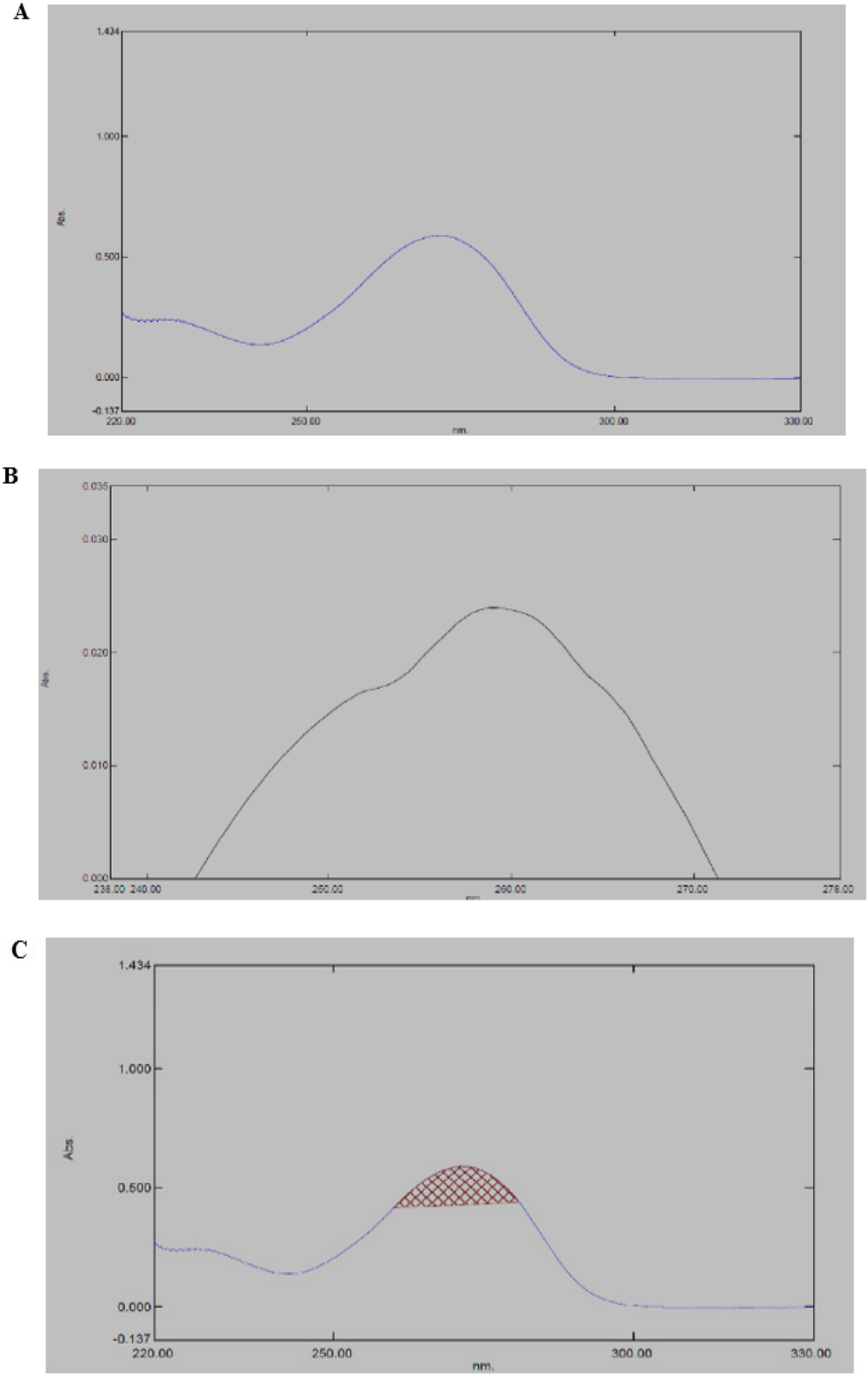
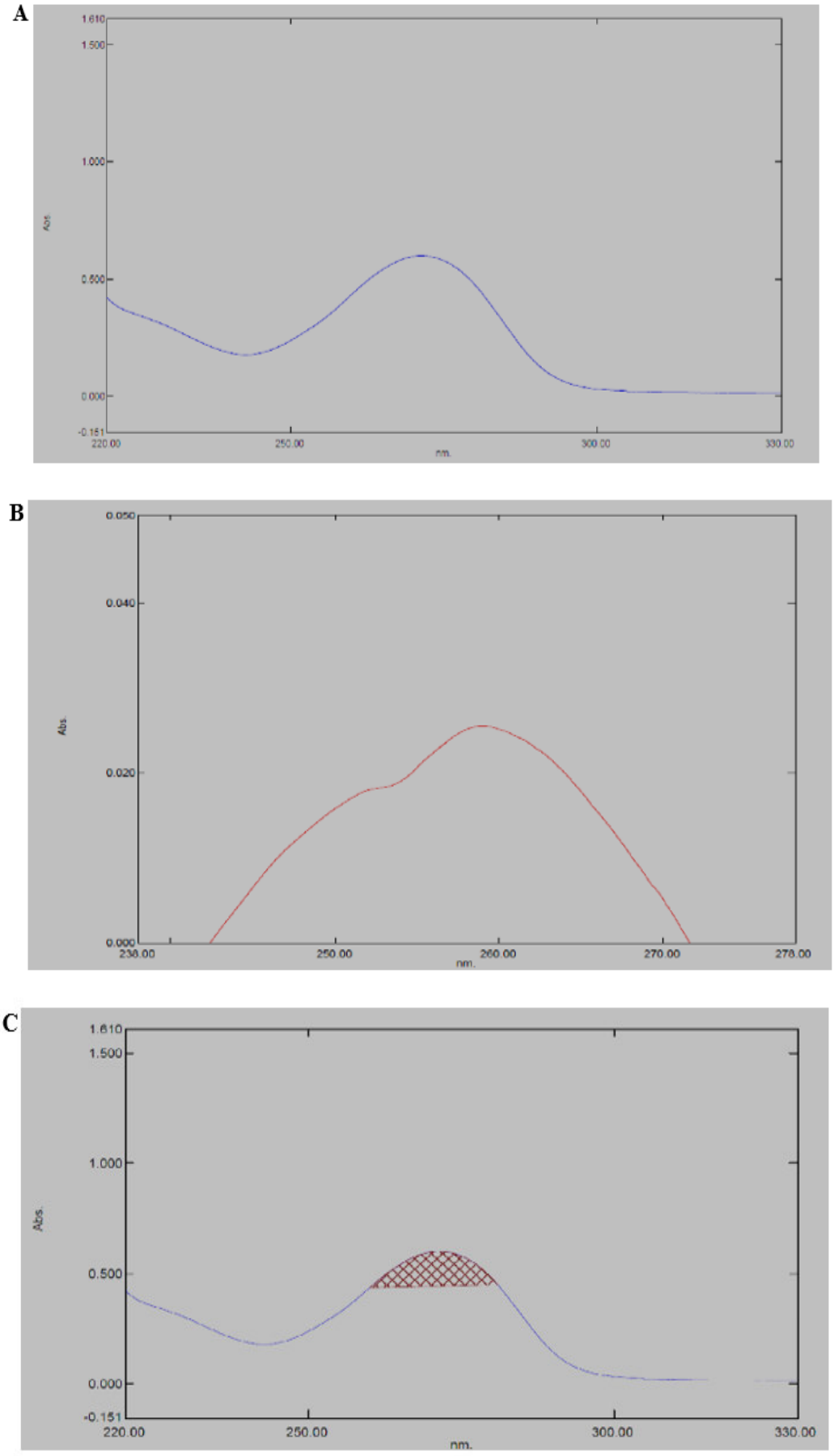
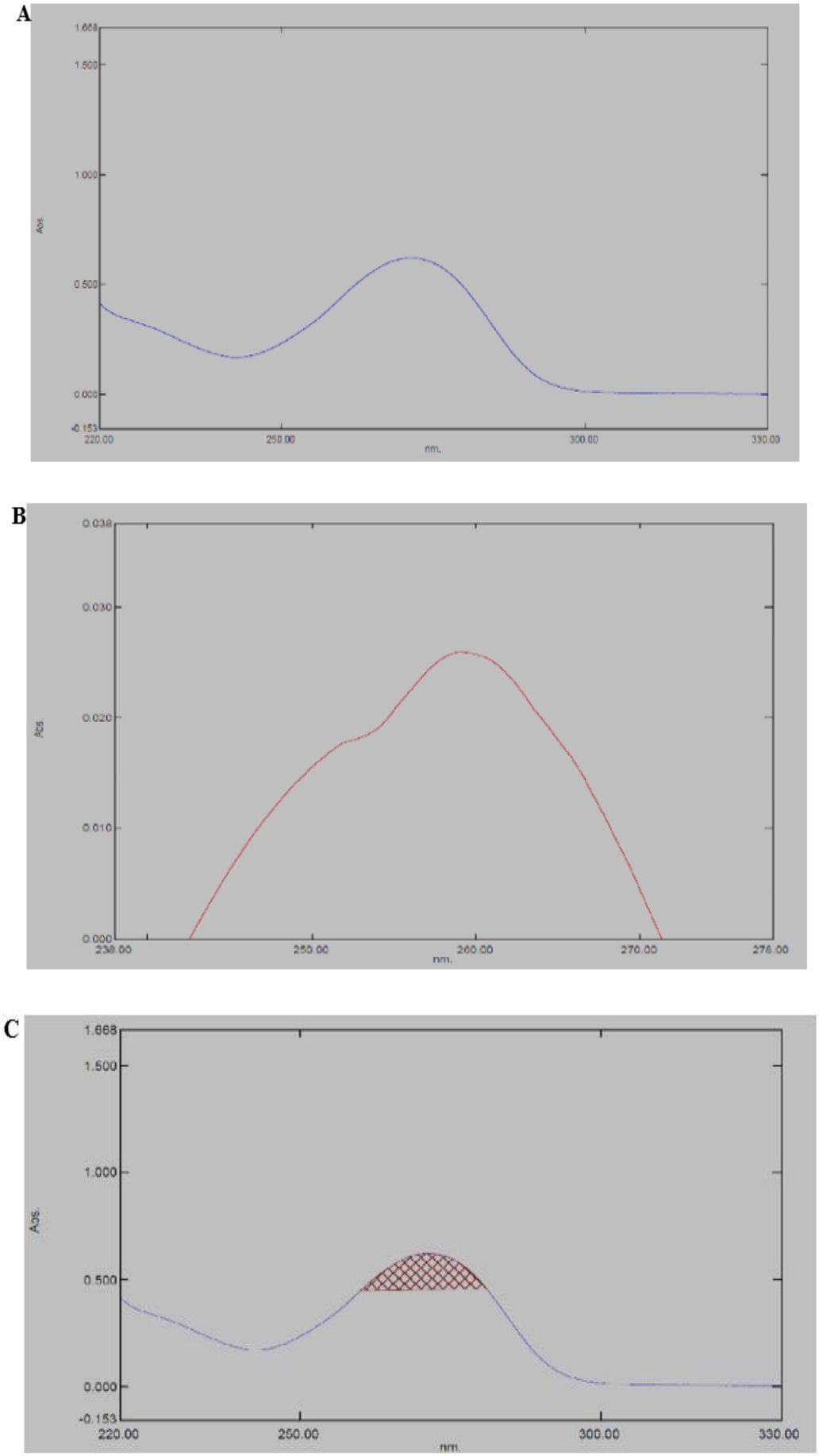


Fig 8: The oxidation stress degradation studies spectrum for methods A, B, and C



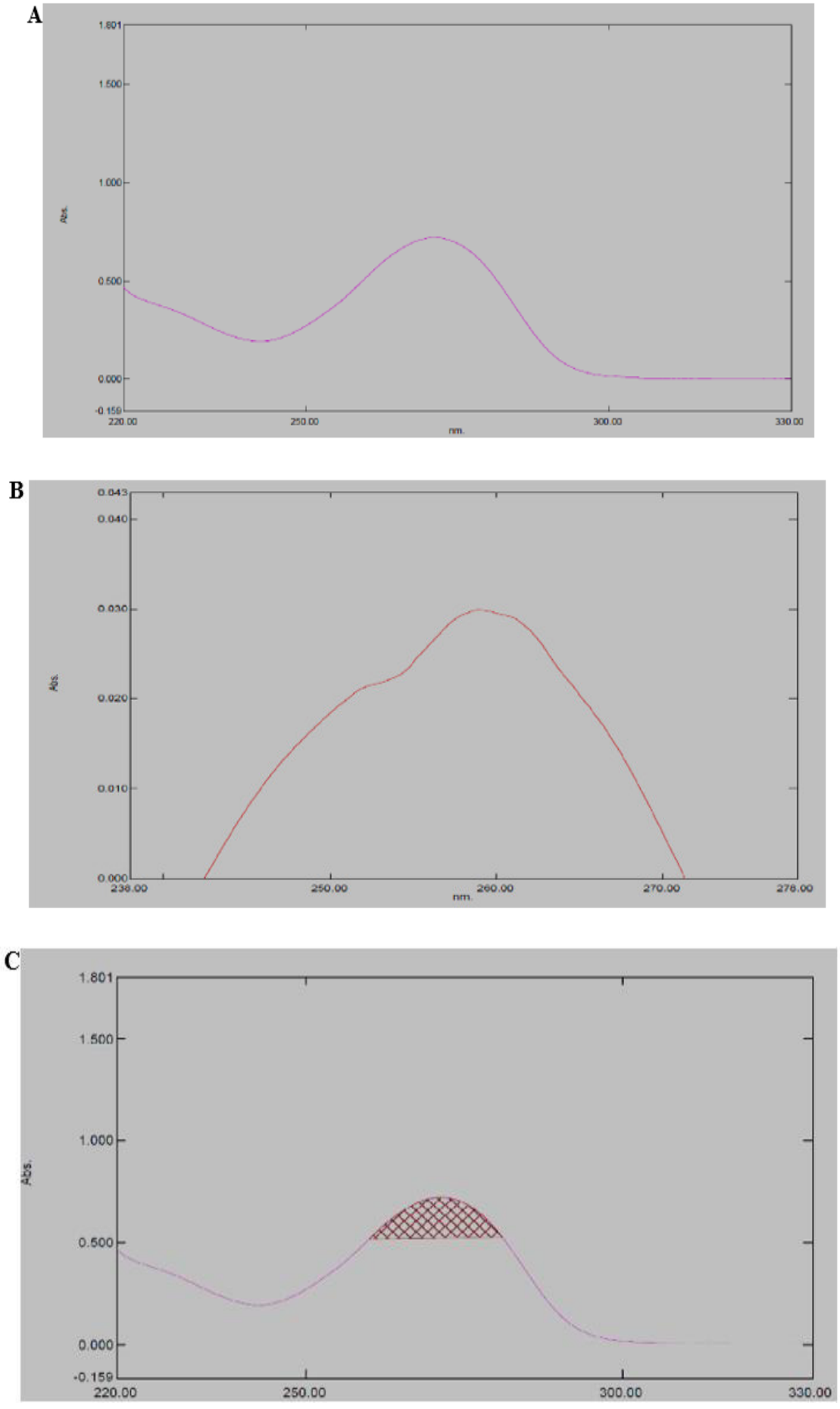
Studies on acid stress degradation indicated that methods A, B, and C exhibited 15.61, 13.33, and 17.12% degradation, respectively (Figure 9).

Fig 9: The acid stress degradation studies spectrum for methods A, B, and C



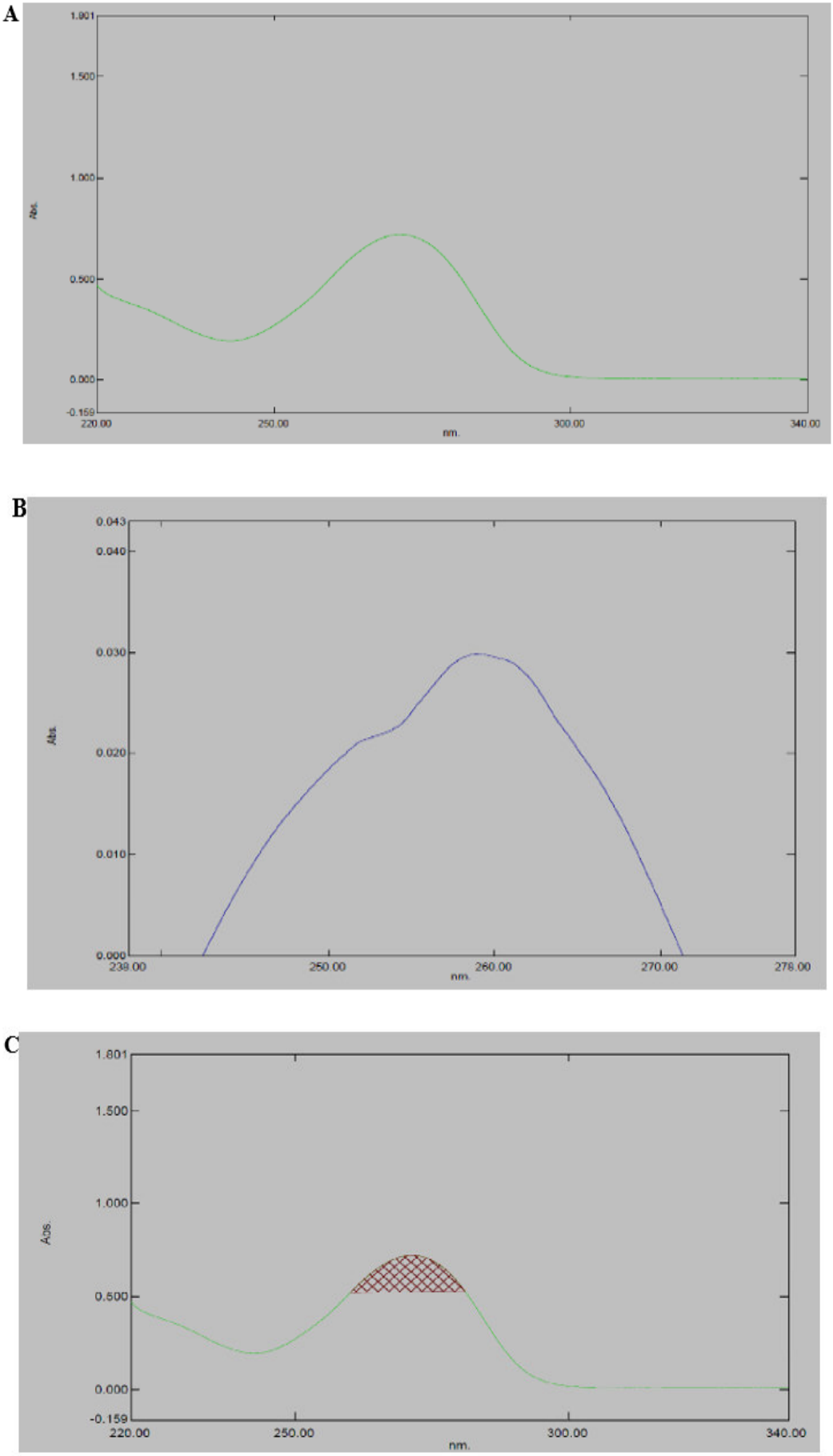
In investigations on alkali stress degradation, it was revealed that methods A, B, and C exhibited degradation rates of 3.09, 3.33, and 6.90%, respectively (Figure 10).

Fig 10: The alkali stress degradation studies spectrum for methods A, B, and C



Dry heat stress degradation studies observed less degradation, with methods A and C found at 1.26 and 2.36%. However, no degradation was seen for method B throughout the analysis period (Figure 11).

Fig 11: The dry heat stress degradation studies spectrum for methods A, B, and C



Regarding photolytic stress degradation, methods A and C showed degradation percentages of 0.28 and 0.40; however, method B showed no degradation at any stage (Figure 12).

Fig 12: The photolytic stress degradation studies spectrum for methods A, B, and C

The research objectives for stress degradation are carried out for methods A, B, and C, listed in Table 7. The obtained % of degradation is significantly good with not numerous variables. Per the ICH and US FDA guidelines, the stress degradation studies are acceptable and can be used for quality control measurements of ritonavir.

Table 7: The desired outcome of ritonavir stress degradation studies employing UV-spectrophotometric

Degradation Condition	Method A	Method B	Method C	% Degradation		
	Absorbance		Area	Method		
				A	B	C
Oxidation	0.588	0.024	2.166	17.29%	20.00%	20.04%
Acid	0.600	0.026	2.245	15.61%	13.33%	17.12 %
Alkali	0.689	0.029	2.522	3.09%	3.33%	6.90%
Dry Heat	0.702	0.030	2.645	1.26%	0%	2.36%
Photolytic	0.709	0.030	2.698	0.28%	0%	0.40%

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), and Method C (Area under the curve spectrophotometric method).

Table 8: Overview of ritonavir UV-spectrophotometric validation parameters

Parameters	Method A	Method B	Method C
λ_{max}	271 nm	258 nm	260-281 nm
Linearity (µg/ml)	10-50 µg/ml	10-50 µg/ml	10-50 µg/ml
Regression coefficient	R^2 = 0.9994	R^2 = 0.9989	R^2 = 0.9999
Regression equation (y=mx+c)	y = 0.0121x + 0.2238	y = 0.0005x + 0.0087	y = 0.0479x + 0.7817
Robustness (% RSD)	0.43-0.76	1.24-1.27	1.03-1.36
LOD (µg/ml)	0.24	0.45	0.38
LOQ (µg/ml)	0.75	1.42	1.14

Method A (Zero order spectrophotometric method), Method B (First-order spectrophotometric method), and Method C (Area under the curve spectrophotometric method).

All validation parameters, like linearity, precision, robustness, accuracy, LOD, and LOQ, were carried out per the ICH guidelines. Table 8 represents the overview of all validation parameters observed by the different spectroscopic methods A, B, and C.

4. DISCUSSION

This experiment describes the analytical validation parameters per the International Conference of Harmonization guidelines[27] to develop a method using UV-Visible spectroscopy. The method is simple, reliable, and selective, providing satisfactory accuracy and precision, lower detection limits, and more specific quantification and sensitivity. Good recoveries were obtained in all cases, and the reliable agreement with the reported procedure[12-18] proved that the proposed method could be applied efficiently for the determination of ritonavir in bulk drug and pharmaceutical dosage forms with satisfactory precision; moreover, the shorter duration of analysis marks the reported method suitable for routine analysis in pharmaceutical dosage forms[34]. The newly developed techniques can distinguish ritonavir in bulk and commercial formulations. Established Methods A (zero-order spectrophotometric approach), B (first-order spectrophotometric method), and C (area under the curve spectrophotometric method), each of which showed excellent results in the study of validation following the ICH guidelines[27]. The ICH guidelines[28] and limitations accept the achieved linearity, accuracy, precision, and robustness. As mentioned above, the linearity investigation of the three approaches reveals a linear curve with an excellent R^2 value (0.99)[35]. In other parameters, such as accuracy, precision, and robustness, the %RSD findings are less than 2%, indicating the usefulness of the methods with ICH criteria[27]. This derivative spectrophotometry also helps to provide and improves the selectivity and sensitivity of determination and gives applicable information in elucidating ritonavir in pharmaceutical formulation[36]. According to assay studies, the ritonavir commercial formulation ritomune (100 mg) contained 99 to 100 % w/v of the drug and showed a %RSD value of less than 2%[37,38]. Studies on stress degradation have shown that minimal degradation was observed during the investigation under various applied stress conditions [39,40].

5. CONCLUSION

The current research proposes an accurate, efficient, and specific routine ritonavir analysis. It can identify related substances or other contaminants during storage conditions and estimate the analyte of interest without interferences. Different methods (such as the Zero Order Spectrophotometric Method, First-order Spectrophotometric, and Area under the Curve Spectrophotometric) can provide a more accurate analysis with validity or enforceability. As a result, according to ICH Q2 (R1) criteria, the UV methods can obtain high specificity in less time while analyzing ritonavir and its formulations. Thus, the results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate, and precise. Therefore, this method can determine ritonavir in bulk or dosage formulations without interference with commonly used excipients and related substances.

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7. AUTHORS CONTRIBUTIONS STATEMENT

Gurram Sai Venkata Nagendra Abhay Raj: Data collection, Data analysis, and interpretation; Sumanta Mondal: Approval of the final version, Revising and editing the manuscript; Subhadip Chakraborty: Presenting the research idea and study design, Supervising the study, Writing the draft of the manuscript; Moumita Ghosh: Data collection, Data analysis.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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