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Force Degradation Study of Ritonavir by RP-HPLC Method

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RP-HPLC,
Ritonavir,
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Validation

ABSTRACT:

Background

Ritonavir is a widely used pharmaceutical compound, and its stability is crucial for its efficacy and safety. Evaluating degradation products and procedure-associated contaminants is essential to ensure the quality of Ritonavir in its dosage form.

Objective

To develop a simple, straightforward, and reproducible ultra-performance liquid chromatographic (UPLC) method for evaluating Ritonavir's degradation products and contaminants in a pharmaceutical dosage form.

Method

- Chromatographic Separation: Performed using Cosmosil –C18 (100 mm x 2.1 ID) 10µ column.
- Elution System: Gradient elution system using a binary mixture of Methanol and Water.
- Flow Rate: 0.8 mL/min.
- Detection: Monitored and detected at 239 nm using a UV-3000M detector.
- Retention Time: Found to be 5.1 minutes.
- Stress Conditions: Ritonavir was subjected to hydrolytic (acid, alkaline, and water), oxidative, photolytic, and thermal stress conditions.
- Validation: Method performance was validated according to the guidelines set forth by the International Conference on Harmonization (ICH).

Results

The developed UPLC method effectively separated Ritonavir and its degradation products, with a retention time of 5.1 minutes. The method proved to be stable and reliable under various stress conditions.

Conclusion

The UPLC method developed is simple, straightforward, and reproducible, making it suitable for evaluating Ritonavir's degradation products and contaminants in a pharmaceutical dosage form, in line with ICH guidelines.

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Introduction

The human immunodeficiency virus is an infection that attacks the immune system (HIV). Acquired immunodeficiency syndrome, or AIDS, is the most severe stage of the ailment. HIV destroys the body's white blood cells, eroding immunity. This elevates the possibility of getting infections, some cancers, and illnesses including TB.

HIV may be transmitted via body fluids from an infected person, including blood, infant milk, semen, and secretions from the genitals. It cannot be spread by snacking together, hugging, or kissing. It may also be passed from a mother to her offspring. HIV is treated and prevented by antiretroviral therapy (ART). If HIV is not treated, it may progress to AIDS, often years later. [1]

Ritonavir (RIT) is chemically known as 5-thiazolylmethyl [(α*S*)-α-[(1*S*, 3S)-1-hydroxy-3-[(2*S*)-2-[3-[(2-isopropyl-4-thiazolyl)methyl]-3-methylureido] methylbutyramido]phenylbutyl]phenethyl]carbamate[2] The medication is marketed by Abbott Laboratories with the trade name NORVIR ,and it is approved by the US-FDA.

RIT is a type of peptidomimetic inhibitor that inhibits the proteases of HIV-1 and HIV-2. It is frequently administered in conjunction with other antiretroviral medications to treat acquired immune deficiency syndrome.

A new drug's substance or product will degrade during forced degradation, which is a more severe mechanism than accelerated deterioration. It is essential to show the specificity of stability-indicating approaches.

It also offers insight into the drug substance's degradation pathways and degradation products, enabling in the understanding of the degradation products' structures. Studies on forced degradation provide information about the chemical behavior of the molecule, which is useful for formulating and packaging designs.

Furthermore, regulatory guidance is highly vague and provides little clarification for how forced degradation tests are performed

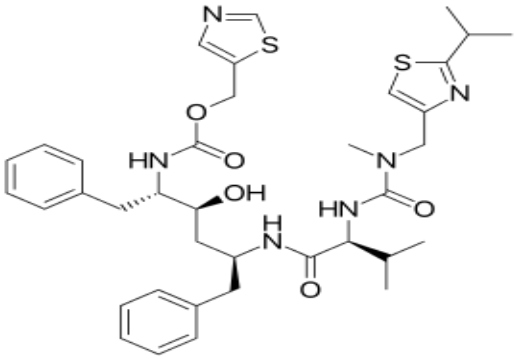

Figure 1: Chemical Structure of Ritonavir

2. Material and Methods

2.1 Equipment

The chromatographic separation was performed on Cosmosil C18 column **(**100 mm x 2.1ID);10µ with UV detector with Agilent ChemStation software. Double beam UV-Visible spectrophotometer for spectroscopic determinations and BioEra's analytical weighing balance was used in the study for weighing.

2.2 Chemicals and Reagents used:

Ritonavir pure drug (API), Ritonavir tablets (Ritovir IP 100 mg), HPLC Grade water, Methanol, Water were purchased from Qualigens, Methanol was procured from Merck Specialties Private Limited.

2.3 Chromatographic conditions

Mobile Phase of Methanol: Water (90:10) was used. The flow rate was 0.8ml/min with injection volume is 20µl. UV Detection wavelength was obtained at 239nm and at room temperature, the separation was accomplished.

2.4 Preparations of solutions

Standard Stock Solution (Preparation)

Accurately weighed quantities (10mg) Ritonavir was dissolved separately in 10ml of solvent (mobile phase) in volumetric flask which gives 1000 ppm solution

Sample Stock Solution (Preparation)

The tablets are selected and weighed and average weight of tablet is calculated. All tablets were grinded into fine powder. **17.31** mg was weighed and dissolved it into 10ml to get 1000 ppm of solution.

3.Result and discussion:

3.1 Selection of Wavelength

UV VIS scan monitored and applied to the solution of Ritonavir was within the specific range of 200-400nm. A wavelength of 239 nm was selected for analysis.

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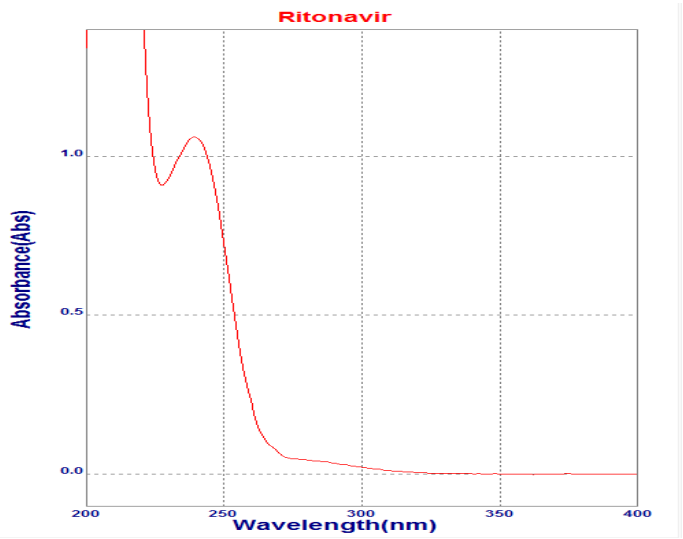

Figure.2: UV spectra of Ritonavir

3.2 Method validation

As per ICH guidelines the following analytical method is validated for its parameters like linearity, accuracy, precision, limit of detection, Limit of quantitation, robustness and system suitability parameters

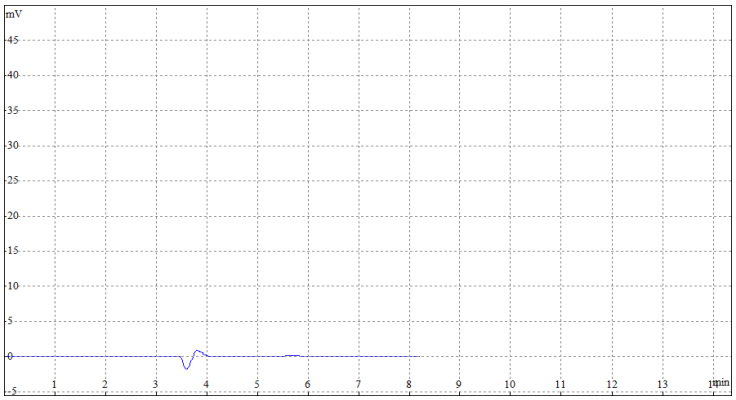

Figure.3: Typical chromatogram of Blank

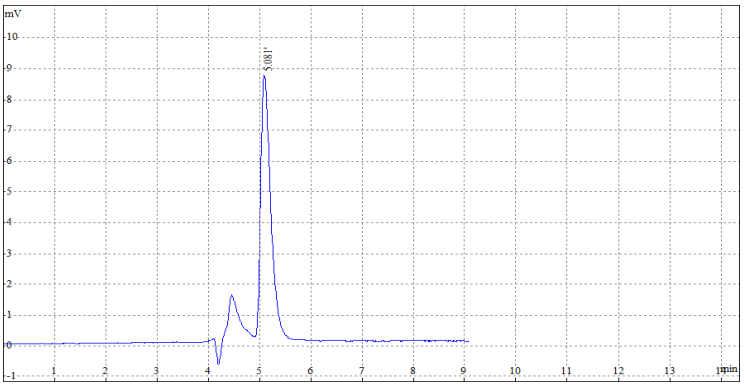

Figure.4: Standard chromatogram of Ritonavir

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3.3 Linearity

Linearity was studied by means of calibration curve using different concentration of drug of 1-5 µg/mL. The correlation coefficient (R^2) for given drug is found to be 0.999 respectively. The calibration curve for given drug were shown in figure.5

Sr .No	Conc.	Area
1	10	499597
2	20	1007836
3	30	1457398
4	40	1914124
5	50	2339954

Table No 1: Linearity of Ritonavir

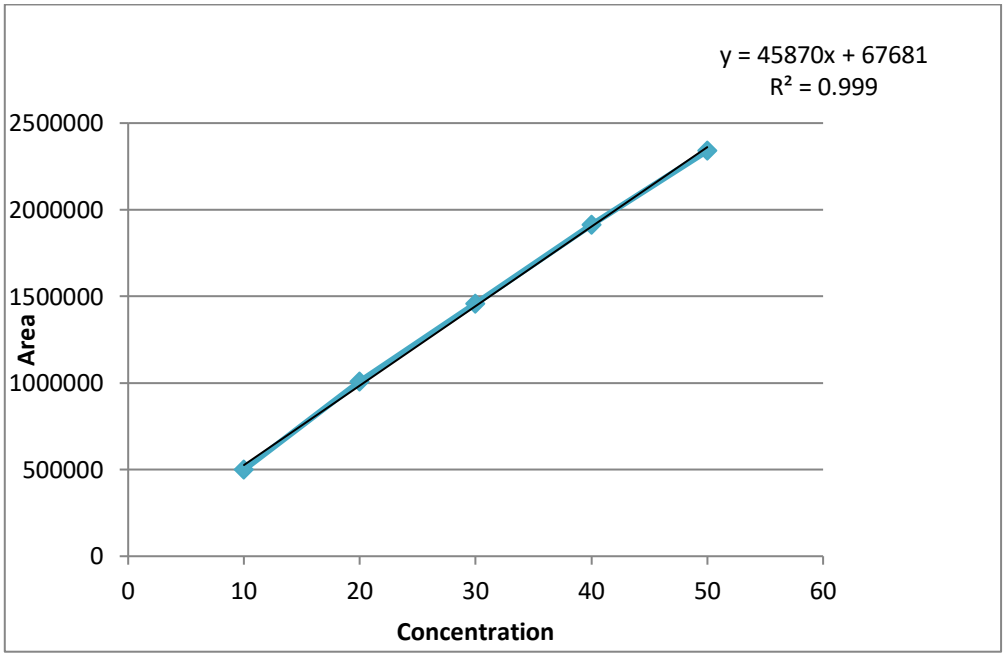

Figure No.5: Calibration curve of Ritonavir

3.4 Precision

Precision were studied by measuring inter-day (by injecting of samples over two consecutive days) and intra-day (repeatability which was carried out by analyzing the drug solutions within same day**).**

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Table No.2: Inter-day study of Ritonavir.

Inter-day			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	30 30 30	1457398 1462605 1460085	1460029.333	2603.9463	0.1783489	0.1897492
2	30 30 30	1450559 1457016 1463576	1457050.333	6508.56792	0.4466948	

Table No.3: Intra-day study of Ritonavir

Intraday			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	30 30 30	1457398 1462605 1460085	1460029.333	2603.9463	0.1783489	0.23828484
2	30 30 30	1459440 1467795 1452779	1460004.667	7523.90858	0.5153346	

3.5 Accuracy

Accuracy of an analytical procedure is closeness of test results to the true value. Accuracy was determined by standard addition method. The study was determined by spiking known amount of standard stock to the test solution prepared from formulation at three different spiking levels. The solution was analyzed for mean recovery and %RSD.

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Sr.no	% Composition	Area of Standard (Area units)	Area of Sample (Area units)	% Recovery	Conc. Taken(ppm)	Conc. Found(ppm)
1	50% Recovery	1457398	1444090	99.08686577	30	29.72605973
2	100% Recovery	1914124	1901907	99.36174459	40	39.74469784
3	150% Recovery	2339954	2328857	99.52575991	50	49.76287995

Table No.4: Recovery study of Ritonavir

3.6 Limit of detection and limit of quantification

The LOD and LOQ values were calculated using the formulas LOD = 0.817 σ/S and LOQ = 0.567 σ/S, respectively. In this method, σ represents the standard deviation of the responses, while S is the mean of the calibration curve slopes**.**

Sr. No.	Drug	SD	Slope	LOD	LOQ
1	Ritonavir	2603.9463	45870	0.1873343	0.5676796

Table No.5: LOD & LOQ study of Ritonavir

3.7 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameter and provides an indication of its reliability during normal usage. The robustness was performed by change in temperature, change in wavelength, and change in PH.

3.7.1 Change in wavelength

Sr. No.	Conc.	Area	Mean	SD	%SD
	20	1007836			
1	20	1003818	1008093	4409.64	0.4374233
	20	1012626			

Table No.6: Robustness study of Ritonavir

3.7.2 Change in Flow rate

Sr. No.	Conc.	Area	Mean	SD	%SD
1	20 20 20	1007836 1017669 1019035	1014847	6109.71	0.602033

Table No.7: Robustness study of Ritonavir

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3.8 Assay procedure

The assay performed by the marketed product (Ritovir IP/100mg). The prepared sample and standard solution were injected into HPLC and peak areas were recorded. Finally, percentage assay of drug was calculated.

Sr. NO.	Conc.	Area of Standard	Area of Sample	% Assay
1	**30ppm**	1457398	1447449	99.31734502

Table No.8: Assay study of Ritonavir

3.9 Forced degradation studies

3.9.1 Acid Degradation.

Ritonavir of concentration of 50ppm was treated with 0.1N HCl at 60ºC for 1hr.

3.9.2 Basic Degradation

Ritonavir 50ppm Treated with 0.1N NaOH at 60ºC for 1hr.

3.9.3 Peroxide Degradation

Ritonavir 50ppm Treated with 0.1N NaOH at 60ºC for 1hr.

3.9.4 Photolytic Degradation

Ritonavir 50ppm Treated with 0.1N NaOH at 60ºC for 1hr.

3.9.5 Thermal Degradation

Ritonavir 50ppm Treated thermally at 60oC for 24hrs.

Sr. NO.	Degradation	Area of Standard	Area of degraded Sample	Degraded upto %	Actual % degradation
1	Acid Degradation	2339954	2083639	89.04615219	10.95384781
2	Base Degradation	2339954	2020949	86.36703969	13.63296031
3	H2O2 Degradation	2339954	2164137	92.48630529	7.513694714
4	Photolytic Degradation	2339954	2317433	99.03754518	0.962454817
5	Thermal Degradation	2339954	2294886	98.07397923	1.926020768

Table No.9 : Degradation study of Ritonavir

4. Conclusion

The present paper described the development of simple, sensitive and accurate analytical methods for the determination of Ritonavir. The procedure presented here does not need any expensive apparatus; therefore, the proposed method can be used advantageously as a routine method for the determination of Ritonavir in quality control and industry, our method may be applied to the determination the content of the cited drugs in commercial tablets.

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5. Abbreviations

RP-HPLC: Reverse Phase High-Performance Liquid Chromatography .
LOD: Limit of Detection.
LOQ: Limit of Quantification.
PPM: Parts per million.
R.T: Retention time
µg/ml: Microgram per milli-liter
ICH: International Conference on Harmonization

6. Acknowledgements

Ethics approval and consent to participate: we ensure the originality and integrity of this article.

Consent for publication: We confirm that all individuals featured in this research article have provided explicit consent for using their data, images, or identities.

Availability of data and material: We pledge to make the data and materials underlying this article available upon request, in compliance with the journal's policies and ethical considerations.

Competing Interests: The authors declare no competing interests that could influence the interpretation or presentation of the review article's findings.

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