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Method Development And Validation For The Simultaneous Determination Of Nirmatrelvir And Ritonavir By Using Rp-Hplc

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Abstract: A simple, rapid, precise, sensitive, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Nirmatrelvir and Ritonavir in pharmaceutical dosage form. Chromatographic separation of Nirmatrelvir and Ritonavir was achieved on Waters Alliance-e2695 by using Waters Symmetry Shield RP-18 (150x 4.6mm, 3.5µ) column and the mobile phase containing Methanol: Heptane Sulfonic acid pH-2.5/OPA in the ratio of 10:90% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 236nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Nirmatrelvir and Ritonavir were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Nirmatrelvir and Ritonavir study of its stability.

Index Terms - HPLC, Nirmatrelvir and Ritonavir.

I. INTRODUCTION

A medication is a chemical utilized to treat illness in humans or animals or to modify the structure of the body [1] in any way.

The ability of pharmaceuticals to treat and cure illness is critical to the advancement of human civilization. Most today's drugs are synthetically produced. These are mass-produced and employed in pharmaceutical formulations for their medicinal properties [2]. These medications, which deliver the medication substance in steady, non-poisonous and tolerable form and ensure its bioavailability and remedial scheme are commonly used to deliver biologically active chemical substances. These formulations include tablets, capsules, ointments, and injectable tablets.

Quality, safety, and efficacy of medications

With the utilize of medications, both safety and efficacy must be considered. In certain cases, drug impurities may have a detrimental influence on the medicine's pharmacological or toxicological properties that outweighs any advantage they provide.

Every nation has a law that establishes criteria and mandatory quality indices for bulk pharmaceuticals and their pharmaceutical formulations. A book called a pharmacopoeia [3,4] is a compilation of these restrictions, which are provided in different articles, journals and particular to each medicine.

New medications are being introduced at an alarming rate in today's market. There are a variety of reasons why certain medications may only be found in a few pharmacopoeias, including the fact that they are either completely novel substances or simply minor structural alterations of existing ones. It's possible that these

medications' standards and testing techniques aren't out of reach under these circumstances. As a result, new analytical methods must be developed for these medications.

Monitoring and managing the release of medicine and the number of contaminants properly is typically the best way to ensure the quality and safety of the pharmaceuticals. As a result, one of the most critical facets of contemporary pharmaceutical analysis is the examination of contaminants in medications. For the separation of mixtures, chromatography is a family of analytical chemistry procedures [5]. There is a "stationary phase" in which the mixture containing the analyte is passed via a channel in which the specimen is in the "mobile phase." Components of the specimen are slowed down by the stationary phase. Components are split in time like marathon runners as they go through the system at various paces. Each part of the system should, in theory, have a certain time it takes to go through it. "Retention time" is the term for this.

One phase is immobile, while another passes across it in a certain direction. To maintain and separate the chemicals, the stationary phase must have some kind of interaction with them.

Types of Chromatography

Depending on modes of chromatography:

Normal Phase HPLC:

The stationary step and mobile step [6] in normal-phase liquid-liquid extraction are both non-polar. Heptane's pure hydrocarbon mobile phase is a good place to start when looking for the best mobile phase. Adding methanol or dioxin to the mobile phase may help enhance the polarity if the sample is heavily retained.

Alcohol/heptane has been used as the mobile phase in oil-soluble vitamin, essential oil, nitro phenol, and other homologous series separations in the normal phase mode. Chiracel OJ and Chiracel OD are chiral separation columns used in normal phase chromatography.

Reverse Phase HPLC:

A polar mobile step, frequently a partly or completely water-based mobile phase, [7-12] is used in reverse phase chromatography, which often employs hydrophobic bonded packing.

Polar compounds elute first in the movable phase. Retention improves with increasing solute hydrophobicity. The greater the eluent strength, the lower the movable phase's polarity should be. Compound classes in the table are eluted in reverse order (thus the name reverse-phase chromatography).

A simple definition of pharmaceutical analysis is the study of drugs. A pharmaceutical, according to Webster's, is a prescription medication. Active pharmaceutical ingredient (API) is the better phrase to use when describing a drug component that has been combined with inert ingredients (excipients) in order to create an acceptable medication for administration to patients. To make sure a new medication product fulfils the set criteria, R&D plays a critical role in its development and follow-up, ensuring that all batches of the drug product are created in accordance with the particular requirements [13,14]. Medicinal assessors in the QC or QA department are accountable for the use of permitted substances and production techniques [15].

Studies on safety and efficacy demanded that medication substance and medication product satisfy 2 crucial conditions.

1. A clear sense of self and purity.
2. Biological availability/dissolution has been established.

II. Materials and Methods

RP-HPLC Simultaneous Method Development for Nirmatrelvir and Ritonavir

a) Equipment:

Table No.7: List of Apparatus utilized in HPLC

S.No	Name	Model	Manufacturer
1.	HPLC	ALLIANCE	Waters e 2695- Empower software2.0versions
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris

4.	Pipettes, beakers and Burettes	-	Borosil
5.	Ultra sonicator	UCA 701	Unichrome
6.	Pump	Isocratic model	--

(b) Reagents & Chemicals

Table No.8: List of chemicals used in HPLC Method

S.No	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Water (Milli Q)	HPLC	In house production
3.	Methanol	HPLC	Rankem
4.	Heptane Sulfonic acid	HPLC	Analytical Reagents
5.	Ortho Phosphoric acid	HPLC	Analytical Reagents

Wavelength (λ_{max}) Determination:

Two medications were estimated at the same time using isobestic wavelength. The wavelength at which the molar absorption of two interconvertible compounds is equal is known as the isobestic point. In order to provide an accurate estimate of two medications, this wavelength was used in simultaneous estimation.

Using a range of 200-400 nm, the PDA Detector was used to scan the maximum absorption wavelength of a drug solution in a combination of Methanol and Heptane Sulfonic acid pH-2.5 adjusted with OPA (10:90) as a blank, and the drug solution in the aqueous solution served as the sample. At 236 nanometers, the absorption curve reaches its isobestic point. So, for the HPLC chromatographic procedure, 236 nm was chosen as the detection wavelength.

Chromatographic conditions:

A large number of trails were run throughout the chromatographic condition selection process before the optimal trail was chosen for the optimized technique.

Standard solution preparation:

Weigh out 150 milligrammes of Nirmatrelvir and 100 milligrammes of Ritonavir as a working standard. Transfer both to a 100-millilitre clean and dry vacuum flask. Add the diluent and sonicate until it dissolves fully. Fill up the flask to the mark using the same solvent. (Stock solution)

Put 5 millilitres of stock solution into a 50-millilitre vacuum flask and fill it up with diluents until it reaches the mark. (150ppm of Nirmatrelvir, 100ppm of Ritonavir)

Sample Solution Preparation:

Transfer 247 milligrammes of Nirmatrelvir and 199milligrammes of Ritonavir sample into a 100-millilitre clean, dry vacuum flask. Before centrifuging for 30 minutes to dissolve the diluent, add it to the mixture and sonicate it for up to 30 minutes. Use the same solvent to get the volume up to the mark. The next step is an injection filter with a pore size of 0.45 microns. (Stock solution)

Put 5 millilitres of stock solution into a 50-millilitre vacuum flask and fill it up with diluents until it reaches the mark (150ppm of Nirmatrelvir, 100ppm a of Ritonavir).

The Nirmatrelvir peak was observed at 2.938 min with peak response 3581428, tailing factor 1.19, Ritonavir peak was observed at 3.886 min, with peak response 2464821, tailing factor 1.07 and resolution 4.79. This trial was optimized.

General preparations

Heptane Sulfonic acid Preparation: 1.80 gm of Heptane sulfonic acid is dissolved in 1 litre of HPLC water, adjust its pH-2.5 with OPA and filter through 0.45µ membrane filter paper.

Movable Phase preparation: Movable phase was prepared by mixing Methanol and Heptane Sulfonic acid pH-2.5/OPA taken in the ratio 10:90. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Chromatographic condition:

Use suitable High-Performance Liquid Chromatographic equipped with PDA detector.

Column	:	Waters Symmetry shield RP-18 (150x4.6 mm, 3.5 µ)
Movable phase	:	Methanol: Heptane Sulfonic acid pH-2.5/OPA (10:90)
Wavelength	:	236 nm
Flow rate	:	1ml/min
Injection volume	:	10µl
Run time	:	6min

Diluent: Acetonitrile is used as a diluent.

Process:

Measure the areas for the Nirmatrelvir and Ritonavir peaks and compute the %Assay using the equations. Inject 10µL of the reference sample into the chromatographic apparatus.

III. METHOD DEVELOPMENT AND METHOD VALIDATION OF HPLC

Optimized Chromatogram:

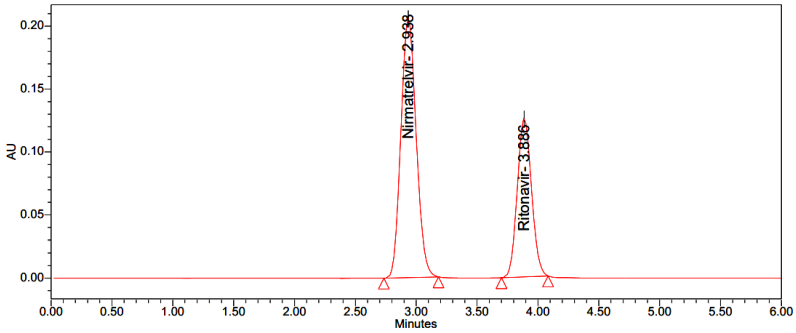

Figure : Optimized chromatogram

Table: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Instrument	Waters HPLC with auto sampler and PDA detector.
Injection volume	10µl
Movable Phase	Methanol: Heptane Sulfonic acid pH-2.5/OPA (10:90)
Column	Waters Symmetry Shield RP-18 (150x 4.6mm, 3.5µ)
Wave Length	236nm
Flow Rate	1 mL/min
Runtime	6min
Temperature	Ambient (25° C)
Isolation Mode	Isocratic mode

System suitability:

The maximum allowable tailing factor for peaks in a standard solution caused by nimatrelvir and ritonavir is 2.0.

Standard solution theoretical plates for Ivacaftor and Lumacaftor peaks should be at least 2000.

In a standard solution, the resolution of the Ivacaftor and Lumacaftor peaks must be at least 2.

Table : System suitability parameters for Nirmatrelvir & Ritonavir

S.NO	PARAMETER	NIRMATRELVIR	RITONAVIR
1	Retention time	2.938	3.886
2	Plate count	12844	15574
3	Tailing factor	1.19	1.07
4	Resolution	----	4.79
5	%RSD	0.34	0.27

Acceptance Criteria: It is required by ICH standards that the plate count be more than 2000, the tailing factor be less than 2, and the resolution be greater than 2. The system's appropriate parameters were all within their respective limitations and passed.

Analytical Method Validation

Precision:

Table: System precision table of Nirmatrelvir & Ritonavir

	Concentration Nirmatrelvir (µg/ml)	Area of Nirmatrelvir	Concentration of Ritonavir (µg/ml)	Area of Ritonavir
1.	150	3581428	100	2464821
2.	150	3596572	100	2453837
3.	150	3560654	100	2449679
4.	150	3582898	100	2466520
5.	150	3590947	100	2457862
6.	150	3578854	100	2455233
Mean	3581892		2457992	
S.D	12320.750		6532.400	
%RSD	0.34		0.27	

Linearity:

Table: Results of linearity for Nirmatrelvir & Ritonavir

S.NO	Nirmatrelvir		Ritonavir	
	Conc.(µg/ml)	Response	Conc.(µg/ml)	Response
1	37.50	846844	25.00	636548
2	75.00	1781815	50.00	1221479
3	112.50	2713037	75.00	1872830
4	150.00	3576514	100.00	2406975
5	187.50	4387252	125.00	3098205
6	225.00	5098609	150.00	3671114
Regression equation	y = 23020.33x +39366.36		y =24460.22x + 9362.43	
Slope	23020.33		24460.22	
Intercept	39366.36		9362.43	
R^2	0.99917		0.99978	

Acceptance criteria: The correlation coefficient (R^2) should be ≥ 0.999 over the specified concentration range. The plot of concentration versus peak area should be linear across the tested range.

Assay:

Table: Assay of Nirmatrelvir & Ritonavir

Brand	Medication	Response	Avg sample area (n=2)	Std. Conc. (µg/ml)	Sample Conc. (µg/ml)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
-	Nirmatrelvir	3521649	3528264	150	150	150	99.9	147.75	98.5
		3534878							
	Ritonavir	2458476	2464957	100	100	100	99.8	100.28	100.3
		2471438							

LOD and LOQ:

LOD refers to Limit of Detection; LOQ refers to Limit of Quantification

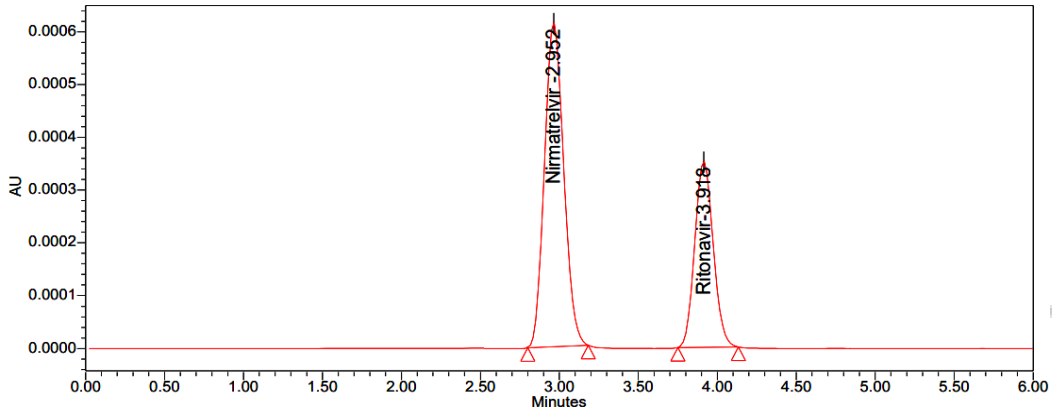

Figure : LOD chromatogram

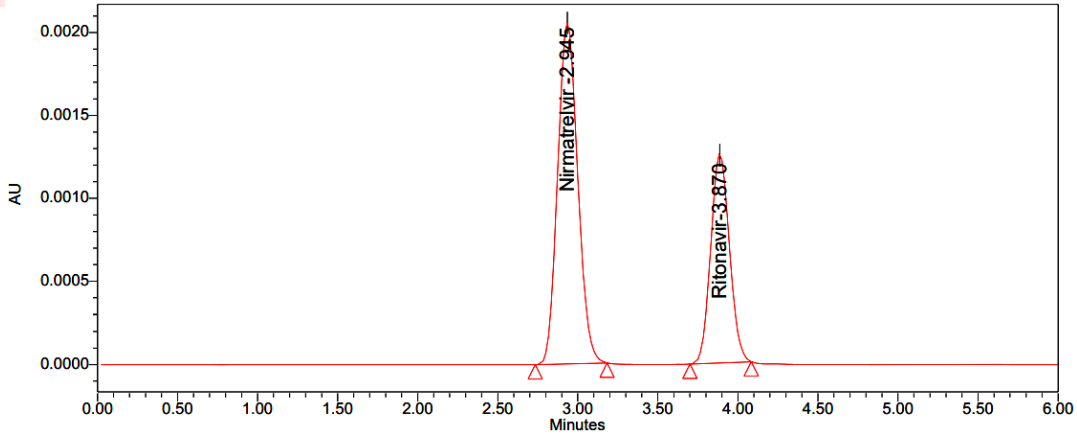

Figure : LOQ chromatogram

Table : Sensitivity parameters (LOD & LOQ)

Medication	LOD(µg/ml)	s/n	LOQ(µg/ml)	s/n
Nirmatrelvir	0.45	3	1.50	10
Ritonavir	0.30	3	1.00	10

Acceptance Criteria: Limit of Detection (LOD) should have a signal-to-noise ratio (S/N) of 3:1.

Limit of Quantitation (LOQ) should have a signal-to-noise ratio (S/N) of 10:1.

Observation: Nirmatrelvir has an LOD of 0.45 µg/mL and LOQ of 1.50 µg/mL, while Ritonavir has an LOD of 0.30 µg/mL and LOQ of 1.00 µg/mL, both meeting the required S/N ratios.

Conclusion: The LOD and LOQ results for Nirmatrelvir and Ritonavir meet the acceptance criteria, indicating that the method is sensitive and capable of detecting and quantifying low concentrations of the drugs accurately.

IV. CONCLUSION

The HPLC approach that was created for the measurement of some pharmaceuticals is quick, easy, accurate, precise, inexpensive, robust, and quick. In addition to being cheap, dependable, sensitive, and easy to prepare, the solvents and mobile phase are also time and labour savers. Laboratories may utilize the sample recoveries for regular medication analysis, and they were in excellent accord with the promises made on the labels. This suggests that the formulation receivers do not interfere with the estimate.

The analysis would benefit most from the proposed techniques, which are concise and easy to understand, as the HPLC method's system validation parameters have shown reliable, accurate, and repeatable finding (without recipient interference, of course).

Results showed that the RP-HPLC stability indicating assay technique was free of interference with degradation products and placebos, and it was also easy to use, accurate, exact, and specific. Which means you may use them to regularly test Nirmatrelvir and Ritonavir.

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