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Research Article

A New Validated Stability Indicating RP-HPLC Method for the Estimation of Nirmatrelvir and its Related Impurities

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Abstract

An efficient and reliable RP-HPLC technique has been developed specifically for the detection and quantification of Nirmatrelvir and its related impurities in pharmaceutical formulations. Waters UPLC Aqcuity system with TUV detector integrated with Empower 2 Software with Agilent C18 column C18 column (5 µm, 4.6 mm X 250 mm) using mobile phase of 0.01N phosphate buffer: Methanol (55:45 v/v) with flow rate 1 ml/min (Detection wavelength 223 nm are the optimised chromatographic conditions for the present study. Degradation studies were performed for Nirmatrelvir drug and its related impurities and the method was validated as per ICH guidelines.

Keywords: Nirmatrelvir; Nirmatrelvir Acid Impurity; Trifluoro Bicycloamide Impurity; Validation; ICH Guidelines

Introduction

Nirmatrelvir (Figure 1) is an antiviral medication. It is an orally active 3C-like protease inhibitor. As evidenced by literature, various analytical methods such as RP-HPLC and UV-spectrophotometric techniques have been explored for the determination of Nirmatrelvir particularly in pharmaceutical formulations. The authors have developed a RP-HPLC method for the estimation of Nirmatrelvir and its two impurities Trifluoro Bicycloamide impurity and Nirmatrelvir acid impurity in tablet dosage forms.

Experimental

Materials and Methods

HPLC grade Methanol, HPLC grade water, Potassium dihydrogen ortho phosphate (AR), Ortho-phosphoric acid (AR) were used for the study.

0.1% Ortho phosphoric acid buffer

1ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

0.01N Phosphate buffer solution

Accurately weighed 1.36 gm of Potassium dihyrogen Ortho phosphate was taken into 1000ml of volumetric flask and about 900 ml of Milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.5 with dil. Ortho phosphoric acid solution.

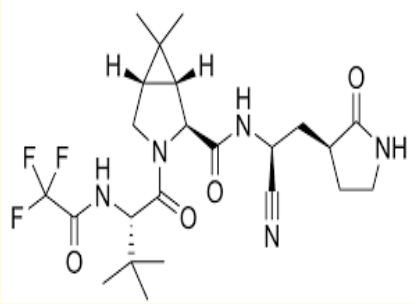


Figure 1: Structure of Nirmatrelvir.

Method validation

Linearity

20 µl of the blank, standard and sample were injected into the chromatographic system and areas for Nirmatrelvir peaks was used for calculation.

Preparation of standard solution

1.5 mg of Nirmatrelvir drug is weighed accurately and transferred to 100ml volumetric flask and dissolved in a small amount of diluent. The solution is sonicated for 15 min and the solution was made up with diluent to 10ml which gives 15 µg/ml solution. 1ml of the above solution was transferred to another 10 ml volumetric flask and was make up with the diluent to give 1.5 µg/ml solution.

Preparation of Nirmatrelvir acid impurity and Trifluoro bicycloamide impurities solution

1.5 mg of Nirmatrelvir Acid impurity and Trifluoro bicycloamide impurity is weighed accurately and transferred to 100 ml volumetric flask and dissolved in a small amount of diluent. The solution is sonicated for 15 min and the solution was made up with diluent to 10ml which gives 15 µg/ml solution. 1ml of the above solution was transferred to another 10 ml volumetric flask and was make up with the diluent to give 1.5 µg/ml solution.

Results and Discussion

Method validation

Linearity

A new RP-HPLC method has been developed proposed for the quantification of Nirmatrelvir and its related impurities using Waters UPLC Aqcuity system with TUV detector integrated with Empower 2 Software with Agilent C18 column C18 column (5 µm, 4.6 mm X 250 mm) using mobile phase of 0.01N phosphate buffer: Methanol (55:45, v/v) with flow rate 1 ml/min (Detection wavelength 223 nm) are the optimised chromatographic conditions (Column temperature 28°C) for the present study. A mixture of water: Methanol (50:50, v/v) is used as diluent. The injection volume is 20 µl and the run time is 12 min. The Limit of detection for Nirmatrelvir is 2.97, whereas limit of quantification for Nirmatrelvir is 9.92 g/ml respectively. The linearity values of Nirmatrelvir and its impurities were shown in Table 1. The chromatogram of Nirmatrelvir and its impurities with the optimised conditions was shown in Figure 2 and the calibration curves of Nirmatrelvir and its impurities were shown in Figure 3.

Table 1: Linearity Study.

% Level	Conc. (µg/mL)	Average peak area		
		Nirmatrelvir	Nirmatrelvir acid impurity	Trifluoro bicyclo amide impurity
25%	0.375	161438	11366	11339
50%	0.75	325252	22912	23090
75%	1.125	476425	34522	34416
100%	1.5	643999	45666	45663
125%	1.875	803326	56733	56591
150%	2.25	964756	68495	67814

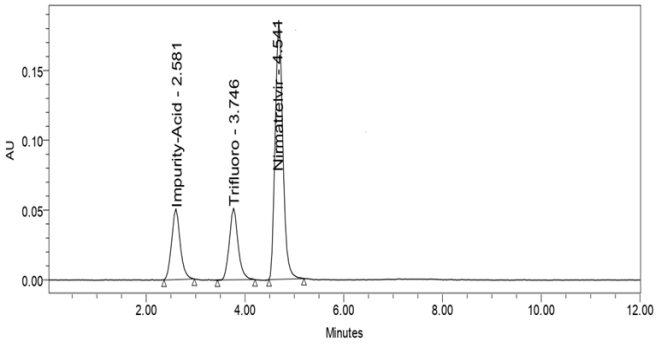
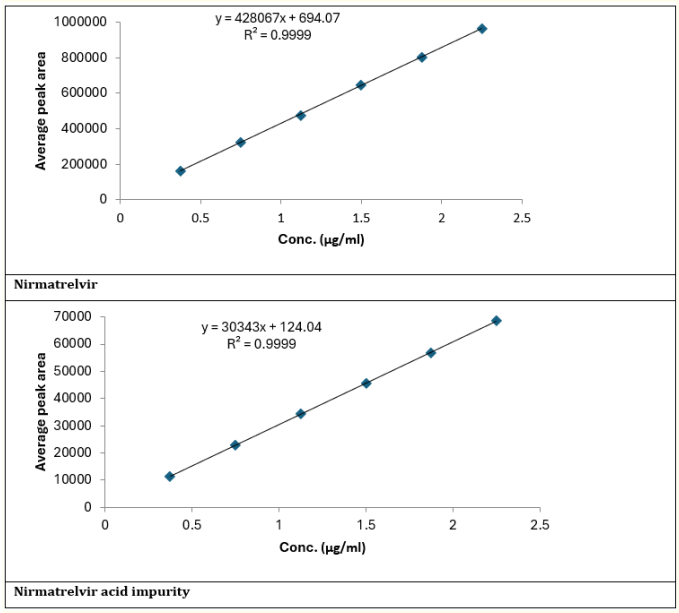


Figure 2: Optimized Chromatogram of Nirmatrelvir and its Impurities.



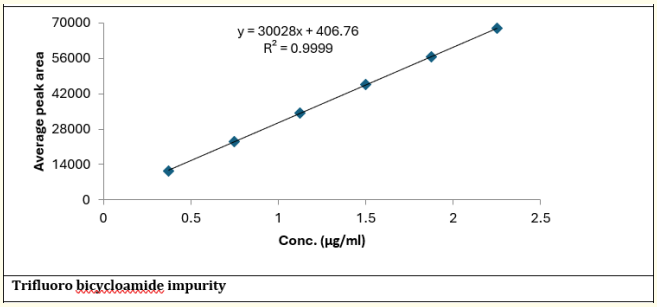


Figure 3: Calibration curves of Nirmatrelvir and its impurities.

Accuracy (50%, 100% and 150% spiking)

No interference from the additives commonly present in the tablets and developed technique constitute to be precise as the percent relative standard deviations for repeatability and intermediate precision is less than 2 as per proposed ICH guidelines (Table 2). For Nirmatrelvir, it indicates that the method has good repeatability. Tablet powder of 15 mg of Nirmatrelvir was weighed and transferred to three different 10ml volumetric flasks labelled 50%, 100% and 150% spiked. 0.5 ml of solution from impurity stock was transferred to the three volumetric flasks labelled 50%, 100% and 150% spiked and made up to 10 ml with diluent.

Table 2: Accuracy study.

Spike level (%)	Nirmatrelvir acid impurity				
	Conc. drug (% w/w)		Recovery (%)	Mean (%)	RSD
	Added	Recovered			
50	0.0492	0.047	95.37	95.36	0.0
	0.0492	0.047	95.37		
	0.0492	0.047	95.33		
100	0.0984	0.098	99.68	99.41	0.3
	0.0984	0.097	99.05		
	0.0984	0.098	99.50		
150	0.1476	0.150	101.41	100.94	0.4
	0.1476	0.149	100.68		
	0.1476	0.149	100.74		
Spike level (%)	**Trifluoro bicycloamide impurity**				
	Conc. drug (% w/w)		**Recovery (%)**	**Mean (%)**	**RSD**
	Added	**Recovered**			
	0.0500	0.048	95.35		
50	0.0500	0.048	95.35	95.35	0.0
	0.0500	0.048	95.35		
	0.0999	0.095	95.58		
100	0.0999	0.095	95.15	95.58	0.4
	0.0999	0.096	96.00		
	0.1499	0.146	97.23		
150	0.1499	0.145	97.07	97.08	0.2
	0.1499	0.145	96.93		

Method precision

Spiking impurity

Tablet powder equivalent to 15mg of Nirmatrelvir is weighed and transferred to a 10ml volumetric flask labelled precision spiked. 1.0ml of solution from impurity stock is transferred to the volumetric flask labelled precision spiked and makeup to 10ml with diluent.

Robustness

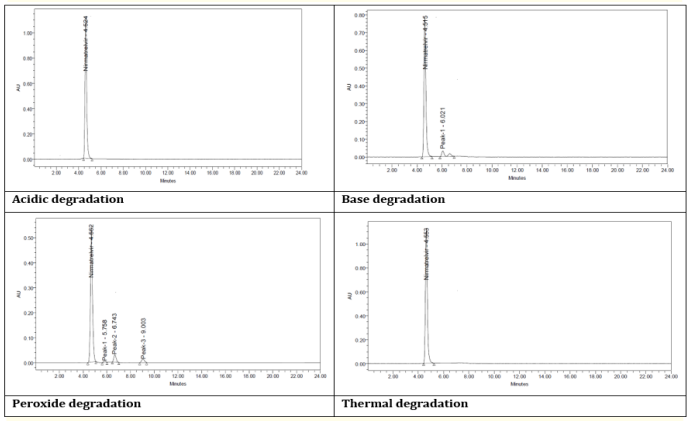
The robustness of this method was determined by assessing the ability to develop a method which remains intact by the small changes in the criteria's such as percent organic content, temperature and flow rate and the relative standard deviation is less than 2.0 indicating that the method is robust (Table 3).

Table 3: Robustness study.

	Flow minus		
S. No	**NRM**	**NRM acid impurity**	**Tri fuoro bicyclo amide impurity**
1	2089575	298545	328754
2	2095774	301776	327666
3	2085545	302755	327556
4	2066656	304645	326444
5	2086766	305734	325644
Avg.	2084863	302691	327213
SD	10918.3	2788.8	1199.0
%RSD	0.5	0.9	0.4
Flow plus			
1	1986545	317565	332556
2	2019656	315465	335545
3	2038455	317655	331456
4	2018676	315345	335656
5	2025465	317686	332656
Avg.	2017759	316743	333574
SD	19147.2	1223.1	1909.5
%RSD	0.9	0.4	0.6
Temperature Minus			
1	2189566	308223	324686
2	2206765	305454	326456
3	2165567	308656	328665
4	2164534	302457	326457
5	2196543	307567	321568
Avg.	2184595	306471	325566
SD	18864.5	2558.9	2643.3
%RSD	0.9	0.8	0.8
Temperature plus			
1	2254667	309756	327856
2	2275656	308677	325677
3	2264555	301665	327897
4	2275465	304667	328666
5	2254556	307677	326545
Avg.	2264980	306488	327328
SD	10477.5	3296.3	1197.0
%RSD	0.5	1.1	0.4

Related substances

The proposed technique was applied for the related substances of commercial formulation consisting 20µL of the blank, standard and sample were injected into the chromatographic system and areas for the Nirmatrelvir and impurities where the peak was used for calculating the % impurity by using the formulae. The % purity of Nirmatrelvir was found to be 99.3. This implies the separation of impurities in tablet dosage form was steady without significant variation.



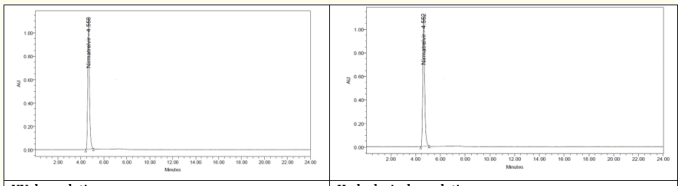


Figure 4: Forced degradation studies.

Forced degradation studies

The guidelines for stability testing of new drugs and products of the ICH require performance tests to be completed in order to elucidate the inherent stability characteristics of the active substance. The purpose of this study was to work out Nirmatrelvir stress degradation studies using the suggested methods, such as acidic, peroxide degradation, alkaline hydrolytic degradation, thermal degradation, UV and hydrolytic degradation and the results were illustrated (Table 4) [1-7].

Table 4: Forced degradation studies.

Degradation Studies	% Recovery*	% Total impurities	% Total found (% w/w)	% Mass balance	Peak purity of Nirmatrelvir	
					Purity angle	Purity threshold
Control	99.73	0.0	99.73	-	-	-
Acid (0.1N HCl at 60°C, 24 Hours)	95.78	0.0	95.78	96.03	0.268	0.442
Base (0.1N NaOH at 60°C for 24 Hours)	92.35	5.38	97.73	97.99	30.958	42.484
Peroxide (3% H_2O_2 at 60°C, 24 Hours)	90.56	7.47	98.03	98.29	0.323	0.371
Thermal (105°C, 6 Hours)	98.76	0.0	98.76	99.02	0.343	0.531
UV ($1.2X10^6$ lux hours and 200.25-watt hours/square meter of UV energy)	99.28	0.0	99.28	99.54	0.254	0.406
Hydrolytic (60°C, 30 mins)	98.41	0.0	98.41	98.67	0.129	0.308

*Mean of three replicates.

Conclusion

The proposed RP-HPLC method for estimation of Nirmatrelvir along with impurities was validated and it is appropriate for routine quantitative analysis of Nirmatrelvir in bulk and its pharmaceutical dosage forms and can be readily accepted for quality control analysis.

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