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A REVIEW OF ANALYTICAL TECHNIQUES USE FOR ESTIMATION OF NIRMATRELVIR AND RITONAVIR IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

This review's objective is to update methodologies for spectrophotometric and chromatographic measurement of Ritonavir and Nirmatrelvir in pharmaceutical dosage forms that are used in viral infection. It implies different analytical procedures like UV, HPLC, RP HPLC, LC-MS, HPTLC, and other methods reported till date. There are numerous articles that describe the analytical process for the same that have been already published. An FDA-approved novel co-packaged drug called nirmatrelvir plus ritonavir has been developed to treat COVID-19. This review provides a summary of the work done on Ritonavir and Nirmatrelvir alone or in combination with or without the presence of impurities and their degradation products.

Keywords: Ritonavir, Nirmatrelvir, Antiviral agent, HPLC, LC-MS

INTRODUCTION:

A combination of Nirmatrelvir and Ritonavir tablets was developed for the treatment and prophylaxis of coronavirus illness. Ritonavir is the HIV-1 protease

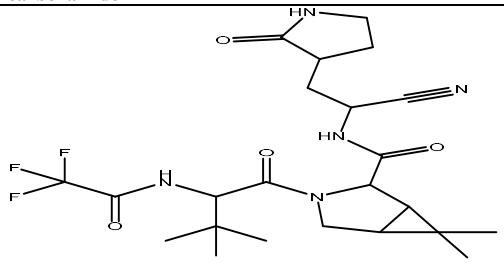
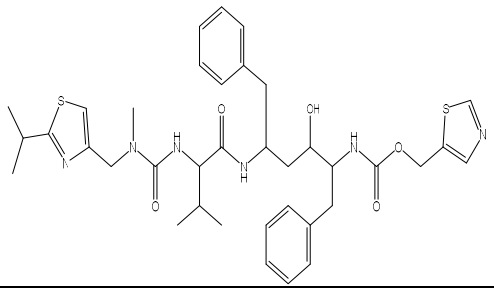
inhibitor and CYP3A inhibitor, while nirmatrelvir is an inhibitor of the SARS-COV-2 **[1]**.

Nirmatrelvir is an inhibitor of the coronavirus 2 that causes severe acute respiratory syndrome. Upon oral administration, it specifically binds to inhibit the M^{pro} targets of SARS-CoV-2. SARS-CoV-2 M^{pro} which having the ability to bind its active site and is responsible for the replication of parasites. This has potent antiviral effects against several human coronaviruses, like SARS-CoV, Middle East respiratory syndrome coronavirus, etc. **[2]**.

Ritonavir is used as a protease inhibitor of HIV-1 and also treat AIDS. Ritonavir is a potent in vitro inhibitor of HIV, it is the causative agent of the acquired immunodeficiency syndrome. Ritonavir extends the half-life of Nirmatrelvir so that it used in combination for the treatment of Viral infection **[3]**.

This combination is recommended for treating mild to moderate cases of Covid-19. Commercially, it is offered as oral tablets containing 100mg of Ritonavir and 150 mg of Nirmatrelvir **[1]**.

DRUG PROFILE:

DRUG NAME	**NIRMATRELVIR**	**RITONAVIR**
IUPAC name	**(1R,2S,5S)-N-[(1S)-1-cyano_2-[(3S)-2-oxopyrrolidin-3-yl]-3-[(2S)-3,3-dimethyl-2-[(2,2,2-trifluoroacetyl) amino] butanoyl]-6,6-dimethyl-3-azabicyclo [3.1.0] hexane-2-carboxamide**	**(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-thiazolylmethyl ester.**
Chemical Structure		
Drug Class	**Antiviral Agent**	**Antiviral Agent**
Category	**Antiretroviral – Protease inhibitors**	**Antiretroviral – Protease inhibitors**
Molecular Formula	**$C_{23}H_{32}F_3N_5O_4$**	**$C_{37}H_{48}N_6O_5S_2$**
Molecular Weight	**499.5 g/mol**	**720.9 g/mol**
Color/Form	**White powder**	**White powder**
Solubility	**DMSO: - 140 mg/ml Ethanol: -50 mg/ml**	**Water: - Practically insoluble Methanol and Methylene Chloride:- Freely soluble Acetonitrile: - Very slightly soluble**
Log P	**2.3**	**3.9**
Half Life ($t_{1/2}$)	**6.05 hr (along with ritonavir)**	**3-5 hr**
Melting point	**190-193˚C**	**120-122˚C**
Boiling point	**742±60˚C**	**947±65˚C**
Density	**1.267±0.06 g/cm^3**	**1.239 ±0.06 g/cm^3**
Mechanism of Action	**Nirmatrelvir can prevent SARS-CoV-2 from replicating by inhibiting M^{pro}**	**It prevents the HIV viral Proteinase enzyme from regularly cleaving the structural and replicative portions that are derived from the main HIV genes**
Therapeutic Uses	**It is used as an Antiviral medication and is given in combination with another Protease Inhibitor to treat Viral Infection**	**It is used for the treatment of HIV infection with another Protease Inhibitor**

Literature Review of Nirmatrelvir and Ritonavir:

SR NO.	DRUG NAME	METHOD	DESCRIPTION	REF. NO.
1.	Nirmatrelvir + Ritonavir	HPLC	Stationary Phase: BDS Hypersil C18 Column Column Size: 250 x 4.6mm (5µm) Mobile Phase: Ethanol: Water (80:20 v/v) Flow Rate: 1ml/min Detection: 215nm Injection Volume: 20µl Concentration Range: 1-20µg/ml Retention Time: Nirmatrelvir = 4.9min Ritonavir = 6.8min Regression Coefficient: Nirmatrelvir = 0.9999 Ritonavir = 0.9998	[6]
2.	Nirmatrelvir + Ritonavir	RP-HPLC	Stationary Phase: Dikma Spursil C18 Column Column Size: 150 x 4.6mm (3µm) Mobile Phase: 0.1 percent Formic acid: Acetonitrile (65:35 v/v) Flow Rate: 1ml/min Detection: 253nm Retention Time: Nirmatrelvir = 4.4min Ritonavir = 2.4min Regression Coefficient: Nirmatrelvir = 0.9992 Ritonavir = 0.9944	[7]
3	Nirmatrelvir + Ritonavir	TLC	Stationary Phase: TLC aluminum Silica gel plates Mobile Phase: Methanol: 2% Urea solution of β-Cyclodextrin: Water (40:5:10 v/v/v) Detection: 215nm Linearity range: 10-50 ng/band Regression coefficient: Nirmatrelvir = 0.9996 Ritonavir = 0.9999	[8]
4.	Nirmatrelvir + Ritonavir	LC-MS/MS	Stationary Phase: Thermo Hypersil GOLD C18 Column Column Size: 2.1 x 100mm (3µm) Mobile Phase: Methanol: 0.1percent Formic Acid-Water (1:1 v/v) Flow Rate: 0.4 ml/min Injection Volume: 2µl Retention Time: Nirmatrelvir = 3.17min Ritonavir = 3.83min Regression Coefficient: Nirmatrelvir = 0.9976 Ritonavir = 0.9925	[9]
5.	Nirmatrelvir + Ritonavir	LC-MS/MS	Stationary Phase: Agilent Poroshell 120 SB-C18 Column Column Size: 2.1x75mm (2.7µm) Mobile Phase: Acetonitrile: 0.1 percent Formic Acid in water (52:48 v/v) Flow Rate: 0.3ml/min Retention Time: Nirmatrelvir = 1.28min Ritonavir = 2.62min Regression Coefficient: Nirmatrelvir = 0.9985 Ritonavir = 0.9956	[10]
6.	Ritonavir + Nirmatrelvir	HPLC	Stationary Phase: Zorbax Eclipse C18 Column Column Size: 4.6 x 250mm (5µm) Mobile Phase: Acetonitrile: Ammonium acetate buffer (50:50 v/v) Flow Rate: 1ml/min Detection: 210nm Injection Volume: 20µl Retention Time: Nirmatrelvir = 3.8min Ritonavir = 6.3min Linearity Range: Nirmatrelvir = 10-200µg/ml Ritonavir = 5-100µg/ml	[11]
7.	Nirmatrelvir + Ritonavir	LC-MS/MS	Stationary Phase: Zorbax XDB C18 Column Column Size: 2.1 x 50mm (3.5µm) Mobile Phase: Ammoniumformate Buffer: acetonitrile (90:10) Flow Rate: 300µl/min Injection Volume: 20µl Retention Time: Nirmatrelvir = 8.2min Ritonavir = 9.2min	[12]
8.	Atazanavir sulphate + Ritonavir	UV - Spectrophotometry 1)Simultaneous determination 2)First order derivative	Solvent: Methanol λ max: 1) Atazanavir sulphate = 249nm, Ritonavir = 239nm 2) Atazanavir sulphate = 254nm, Ritonavir = 264nm Concentration: Atazanavir sulphate = 5-50µg/ml Ritonavir = 10-90µg/ml Regression Coefficient: 1) Atazanavir sulphate = 0.9988	[13]

			Ritonavir = 0.9996 **2) Atazanavir sulphate = 0.9994** **Ritonavir = 0.9978**	
9.	**Ritonavir**	**UV-Vis Spectrophotometry**	**Solvent: Methanol** **λ max: 246nm** **Concentration: 10-30µg/ml** **Regression Coefficient: 0.996**	**[14]**
10.	**Lopinavir + Ritonavir**	**RP-HPLC**	**Stationary Phase: Phenomenex Gemini C18 Column** **Column Size: 250 x 4.6mm (5µm)** **Mobile Phase:** **Potassium Hydrogen Phosphate Buffer: Acetonitrile: Methanol (50:35:15 v/v/v)** **Flow Rate: 1ml/min** **Detection: 254 nm** **Retention Time: Lopinavir = 6 ± 0.2min** **Ritonavir = 3.7 ± 0.1min** **Regression Coefficient: Lopinavir = 0.9999** **Ritonavir = 0.9998**	**[15]**
11.	**Indinavir + Nelfinavir + Ritonavir + Saquinavir**	**HPLC**	**Stationary Phase: Keystone Beta Basic C4 Column** **Column Size: 250 x 3mm (5 µm)** **Mobile Phase:** **Acetonitrile 50: Ammonium Formate Buffer (52:48)** **Flow Rate: 0.5ml/min** **Detection: Indinavir, Nelfinavir, Ritonavir = 218nm** **Saquinavir = 235nm** **Retention Time: Indinavir = 0.85min** **Nelfinavir = 2.51min** **Ritonavir = 2.85min** **Saquinavir = 1.70min**	**[16]**
12.	**Lopinavir + Ritonavir**	**RP-HPLC**	**Stationary Phase: X-Bridge C18 Column** **Column Size: 150 x 4.6mm (5µm)** **Mobile Phase:** **Acetonitrile: Potassium Dihydrogen Phosphate Buffer: Methanol (50:40:10)** **Flow Rate: 1.1ml/min** **Detection: 220nm.** **Injection Volume: 20µl** **Retention Time: Lopinavir = 6.6min** **Ritonavir = 5.7min** **Regression Coefficient: Lopinavir = 0.99991** **Ritonavir = 0.99995**	**[17]**
13.	**Lopinavir + Ritonavir**	**RP-HPLC**	**Stationary Phase: Phenomenex Luna C18 Column** **Mobile Phase:** **Acetonitrile: Triethylamine (67:33 v/v)** **Flow Rate: 1.2ml/min** **Detection: 240 nm** **Retention Time: Lopinavir = 9.1 ± 0.0148min** **Ritonavir = 8.2 ± 0.0217min** **Regression Coefficient: Lopinavir = 0.998** **Ritonavir = 0.999**	**[18]**
14.	**Ritonavir Sulfate**	**HPLC**	**Stationary Phase: Phenomenex C18 Column** **Column Size: 250 x 4.6mm (5µm)** **Mobile Phase:** **Acetonitrile: Phosphoric Acid (55:45 v/v)** **Flow Rate: 1ml/min** **Detection: 210nm** **Injection Volume: 20µl** **Concentration: -1.0mg/ml** **Retention Time: 6min**	**[19]**
15.	**Ritonavir**	**HPLC**	**Stationary Phase: Zorbax Eclipse XDB C18 Column** **Column Size: 100 x 4.6mm (3.5µm)** **Mobile Phase: Acetonitrile: MilliQ Water (55:45 v/v)** **Flow Rate: 0.75ml/min** **Detection: 210 nm** **Injection Volume: 20µl** **Concentration: - 0.12-125µg/ml** **Retention Time: 5.2min**	**[20]**
16.	**Ritonavir**	**LC-MS/MS**	**Stationary Phase: Atlantis Premier BEH C18 AX with VanGuard 108 FIT Column** **Column Size: 150 x 2.1mm (5µm)** **Mobile Phase:** **Formic Acid in Methanol: Formic Acid in Ammonium formate (0.2:0.2 v/v)** **Retention Time: 15min**	**[21]**
17.	**Docetaxel + ritonavir**	**RP-HPLC**	**Stationary Phase: Zorbax eclipse plus C18 Column** **Column Size: 150 x 4.6mm (3.5µm)** **Mobile Phase: Acetonitrile: water (60:40 v/v)**	**[22]**

			Flow Rate: 1ml/min **Detection: 239nm** **Injection Volume: 25µl** **Retention Time: Docetaxel = 7.2 ± 0.22min** **Ritonavir = 8.3 ± 0.27min**	
18.	**Atazanavir + Darunavir + Ritonavir**	**UPLC-MS/MS**	**Stationary Phase: BEH C18 Column** **Column Size: 50 x 2.1mm (1.7µm)** **Mobile Phase: Ammonium formate: acetonitrile** **Flow Rate: 0.300ml/min** **Detection: 239nm** **Retention Time: Atazanavir = 0.69min** **Darunavir = 1.02min** **Ritonavir = 1.54min**	**[23]**
19.	**Paritaprevir + Dasabuvir + Ritonavir + Ombitasvir**	**HPLC-DAD**	**Stationary Phase: Reverse phase waters symmetry C18 Column** **Column Size: 150 x 4.5mm (3.5µm)** **Mobile Phase:** **Acetonitrile: Dihydrogen Orthophosphate (65:35 v/v)** **Flow Rate: 1ml/min** **Detection: 254nm** **Injection Volume: 20µl** **Retention Time: Paritaprevir = 1.476min** **Dasabuvir = 2.835min** **Ritonavir = 3.499min** **Ombitasvir = 6.388min**	**[24]**
20.	**Lopinavir + Ritonavir**	**HPLC**	**Stationary Phase: Agilent TC C18 Column** **Column Size: 250 x 4.6mm (5µm)** **Mobile Phase:** **Acetonitrile: Phosphoric Acid (55:45 v/v)** **Flow Rate: 1.2ml/min** **Detection: 240nm** **Retention Time: Lopinavir = 6.68min** **Ritonavir = 4.35min**	**[25]**
21.	**Atazanavir + ritonavir**	**1)Ratio Derivative Method** **2)Area Under Curve Method**	**λ max: - 1) Atazanavir = 280.01nm** **Ritonavir = 286.12nm** **2)Atazanavir = 246.97-252.03nm** **Ritonavir = 240.78-244.16nm** **Concentration range: Atazanavir = 15-75µg/ml** **Ritonavir = 5-25µg/ml**	**[26]**
22.	**Paritaprevir + Ombitasvir + Ritonavir**	**HPLC**	**Stationary Phase: Intersil ODS C18 Column** **Column Size: 250 x 4.6mm (5µm)** **Mobile Phase:** **Ammonium Dihydrogen Phosphate: Acetonitrile: Methanol (60:20:20 v/v/v)** **Flow Rate: 1ml/min** **Detection: 230nm** **Injection Volume: 20µl** **Retention Time: Paritaprevir = 12.8min** **Ombitasvir = 7.8min** **Ritonavir = 5.7min**	**[27]**
23.	**Lopinavir + Ritonavir + Efavirenz**	**HPLC**	**Stationary Phase: NovaPak C18 Column** **Column Size: 100 x 8mm (4 µm)** **Mobile Phase:** **Tetramethylammonium perchlorate: Acetonitrile: Methanol (50:45:5 v/v/v)** **Flow Rate: 1.5ml/min** **Detection: 205nm** **Injection volume: 25µl** **Retention Time: Lopinavir = 15min** **Ritonavir = 8min** **Efavirenz = 13min**	**[28]**
24.	**Ritonavir**	**RP-HPLC**	**Stationary Phase: Symmetry C18 Column** **Column Size: 100 x 4.6mm (3.5µm)** **Mobile Phase:** **Acetonitrile: Phosphate Buffer (50:50 v/v)** **Flow Rate: 1ml/min** **Detection: 239nm** **Injection Volume: 20µl** **Retention Time: 5.10min** **Regression Coefficient: 0.9998**	**[29]**
25.	**Ritonavir + Lopinavir + Indinavir**	**UPLC-MS/MS**	**Stationary Phase: Acquity UPLC BEH C18 Column** **Column Size: 2.1 x 50mm (1.7µm)** **Mobile Phase:** **Methanol: 0.1 percent Formic Acid (60:40 v/v)** **Flow rate: 0.200 ml/min** **Retention Time: Ritonavir = 1.54min** **Lopinavir = 1.71min** **Indinavir = 0.63min**	**[30]**

26.	**Ritonavir**	**RP-UPLC**	**Stationary Phase: Acquity UPLC BEH Shield RP-18 Column Column Size: 100 x 2.1mm (1.7µm) Mobile Phase: Potassium Dihydrogen Phosphate Buffer: Acetonitrile (80:20 v/v) Flow Rate: 0.5ml/min Detection: 240 nm Injection Volume: 3µl Retention Time: 10.5min**	**[31]**
27.	**Valacyclovir hydrochloride + Ritonavir**	**RP-HPLC**	**Stationary Phase: Agilent TC C18 Column Column Size: 250 x 4.6mm(5µm) Mobile Phase: Acetonitrile: Methanol: Water (41.5:35:23.5 v/v/v) Flow Rate: 1.3ml/min Detection: 222nm Injection Volume: 20µl Retention Time: Valacyclovir hydrochloride = 2.61min Ritonavir = 5.64min Regression Coefficient: Valacyclovir hydrochloride = 0.997 Ritonavir = 0.996**	**[32]**
28.	**Darunavir + Ritonavir + Emtricitabine + Tenofovir**	**HPLC**	**Stationary Phase: Kromasil C18 Column Column Size: 50 x 4.6mm (5µm) Mobile Phase: Sodium Dodecyl Sulphate: 1-Pentanol (97.5:2.5 v/v) Flow Rate: 1ml/min Detection: 214nm Injection Volume: 20µl Retention Time: Darunavir = 8.2min Ritonavir = 18.4min Emtricitabine = 3.6min Tenofovir = 5.5min Regression Coefficient: Darunavir = 0.9996 Ritonavir = 0.9991 Emtricitabine = 0.998 Tenofovir = 0.995**	**[33]**
29.	**Paritaprevir + Ritonavir + Ombitasvir**	**1) HPLC 2) HPTLC**	**Stationary Phase: 1) RP-C18 Kinetix core-shell Column 2) Aluminum HPTLC Plates coated with Silica gel 60F254 Column Size: 1) 150 x 4.6mm (5µm) 2) 20 x 10cm Mobile Phase: 1)Sodium Dihydrogen Phosphate: Ethanol (56:44 v/v) 2)Methylene Chloride: Ethyl Acetate: Methanol: Ammonia (5:3:1:1 v/v/v/v) Flow Rate: 1)1ml/min Detection: 1)254nm 2)243nm Injection Volume: 1)20µl 2)10µl Retention Time: 1) Paritaprevir = 2.3 ± 0.1min Ritonavir = 4.5 ± 0.1min Ombitasvir = 5.5 ± 0.1min 2) Paritaprevir = 0.2 ± 0.1min Ritonavir = 0.6 ± 0.1min Ombitasvir = 0.7 ± 0.1min**	**[34]**
30.	**Lopinavir + Ritonavir**	**LC**	**Stationary Phase: LiChrospher 100 RP 18 Column Column Size: 250 x 4.6mm (5µm) Mobile Phase: Acetonitrile: Methanol: Water (53:10:37 v/v/v) Flow Rate: 1ml/min Detection: 210nm Retention Time: Lopinavir = 9.8min Ritonavir = 8min**	**[35]**

CONCLUSION:

Various methods for the determination of Nirmatrelvir and Ritonavir have been reported. Present research articles found to determine mostly RP-HPLC assay methods and LC-MS/MS methods used for the estimation of Nirmatrelvir and Ritonavir. By the survey of research articles, the determination of Nirmatrelvir and Ritonavir single or in combination with other drugs

like lopinavir, darunavir, emtricitabine, tenofovir, indinavir, efavirenz, atazanavir, etc. in pharmaceutical dosage forms.

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