



## A Review on Analytical Methods for Estimation of Antiviral Drugs in Bulk and in Pharmaceutical Dosage Forms

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### ABSTRACT

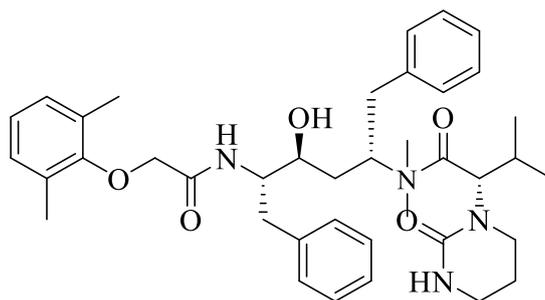
Accurate estimation of antiviral drugs in bulk and pharmaceutical dosage forms is an imperative task for the assurance of their quality, safety, and efficacy. This review focuses on various analytical methods developed for the quantitative determination of major antiviral drugs such as Lopinavir, Ribavirin, Peramivir, Fostemsavir, Molnupiravir and Remdesivir. The techniques that were discussed included UV-Visible spectrophotometry, HPLC, UPLC, HPTLC, and advanced hyphenated techniques such as LC-MS/MS on their principles, sensitivity, precision, and applicability. The method development parameters based on ICH guidelines have been highlighted covering linearity, limit of detection, recovery, and robustness studies. Among the different techniques, HPLC and LC-MS/MS were found to be the most reliable for high accuracy and trace-level detection, while UV and HPTLC remained viable economical options for routine analysis. This article reviews the current state of various analytical approaches employed for the estimation of antiviral drugs, along with their advantages, shortcomings, and applicability in pharmaceutical quality control and research studies.

**Keywords:** Antiviral drugs, analytical methods, HPLC, LC-MS/MS, UV spectrophotometry, HPTLC, method validation, ICH guidelines.

### INTRODUCTION:

#### LOPINAVIR

Lopinavir is a man-made antiretroviral agent classified as an HIV-1 protease inhibitor. It inhibits the viral protease enzyme, which is required to cleave viral polyproteins into active proteins. This inhibits viral replication.<sup>1</sup> Lopinavir was initially created for the treatment of HIV-1 infection. It became popular during the COVID-19 pandemic.<sup>2</sup> The IUPAC name of lopinavir is (2S)-N-[(2S,4S,5S)-5-[2-(2,6-Dimethylphenoxy) acetamido]-4-hydroxy-1,6-diphenyl hexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide. Scientists explored its possible antiviral action against SARS-CoV-2, but later clinical trials revealed it had minimal therapeutic value.<sup>3</sup> To determine its quality, safety, and efficacy, various methods have been established to quantify it in bulk drug, pharmaceutical formulations, and biological fluids. Some common methods are High-Performance Liquid Chromatography (HPLC)<sup>4</sup> for standard quality control and stability testing, Liquid Chromatography-Mass Spectrometry (LC-MS/MS) for analytical applications requiring sensitivity, UV-Visible spectrophotometry for rapid and inexpensive testing, and chromatographic procedures under stability-indicating conditions for degradation profiling under stress conditions.<sup>5</sup> These have proven vital for pharmacokinetic investigation, quality control, and regulatory compliance, particularly while assessing its application for COVID-19.


**Figure 1: Lopinavir Structure**
**TABEL-1: Methods for determination of Lopinavir Single and drugs by UV Spectroscopy, Chromatography and other techniques**

S.NO	METHOD	DESCRIPTION	REFERENCE
1.	HPLC-UV	Mobile phase: cold acetonitrile and liquid-liquid extraction with <i>n</i> -hexane-ethyl acetate (7:3, v/v). Column: Phenomenex Gemini column (C <sub>18</sub> , 150 mm × 2.0 mm, 5 μm) Temp required: 40°C wave length: 211 nm linear range: 10–10,000 ng/mL Flow rate: 0.30 mL/min Retention time: 5.8±0.2 min LLOQ: ± 20% Correlation coefficient(r <sup>2</sup> ): 0.99 RSD: ≤15%	6
2.	UHPLC-MS	Mobile phase: Acetonitrile and water containing 0.1% formic acid Column: Agilent ZORBAX eclipse plus C <sub>18</sub> (2.1 mm × 50 mm, 1.8 μm) column Temp required: 35 °C. Flow rate: 0.4 mL/min Linear range: 5–500 ng/mL LLOQ: ± 20% Correlation coefficient(r <sup>2</sup> ): 0.9984 Intra-day and inter-day precision: ±15%. RSD: ≤20%	7
3.	RP-HPLC	Mobile phase: potassium hydrogen phosphate buffer (PH adjusted to 6.0±0.1 with diluted potassium hydroxide solution), acetonitrile and methanol (50:35;15 v/v) Column: Phenomenex geminiC18 (250mm x 4.6mm,5μ) Temp required: 35 °C Wave length: 254nm. Flow rate: 1 mL/min Retention time: 6.0±0.2 min Linear range: 400–600μg/mL LOQ:103 μg/mL LOD: 34 μg/mL Correlation coefficient(r <sup>2</sup> ): 0.9984 RSD: ≤20%	8
4.	LC-MS	Mobile phase: 80:20 v/v ACN and 0.1% HCOOH in water Column: Acquity BEH C18 column Temp required: 35 °C Flow rate: 0.30 mL/min Retention time: 6.0±0.2 min	9

		Linear range: 10-150 ng/mL LOQ: 0.010 g/mL LOD: 0.0003 g/mL Correlation coefficient( $r^2$ ): 0.999 RSD: 1.92%-1.33%	
5.	HPLC	Mobile phase: Acetonitrile, Methanol and Tetramethyl ammonium perchlorate (TMAP) in dilute aqueous trifluoroacetic acid (TFA) Solvent: 45:5:50 (v/v/v) of acetonitrile: methanol:0.02 M TMAP in 0.2% TFA Linearity range: 0.060 to 24.06 $\mu\text{g/mL}$ Precision: Intra-day and Inter-day precision: % RSD 1.5-4.0%	10
6.	LC	Mobile phase: Acetonitrile-Water-Methanol (53:37:10, v/v/v) Flow rate: 1 mL/min Wavelength: 210nm Linearity range: 40-360 $\mu\text{g/mL}$ Precision: Intra-day and Inter-day precision: % RSD < 0.70%	11
7.	RP-HPLC	Mobile phase: Acetonitrile, Triethylamine (0.5%) pH 5.0 adjusted with glacial acetic acid, (67:33) %v/v Linearity range: 40-200 $\mu\text{g/mL}$ Correlation coefficient: 0.999 Retention time: 8.2 min % RSD: 0.3499 LOD: 40 $\mu\text{g/mL}$ LOQ: 160 $\mu\text{g/mL}$ Precision: Intra-day precision: % RSD 0.0456 Inter-day precision: % RSD 0.0647	12

## RIBAVIRIN

Ribavirin is a broad-spectrum, synthetic antiviral nucleoside analogue used extensively for the treatment of viral infections like hepatitis C, respiratory syncytial virus (RSV), and some viral haemorrhagic fevers.<sup>13</sup> As a result of its clinical importance, there is a need for precise and reliable analytical techniques to determine ribavirin levels in drug formulations and biological samples for activities like quality control, pharmacokinetic analysis, and therapeutic monitoring of drugs. Its IUPAC name is 1- $\beta$ -D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide. One of the most widely utilized methodologies is Reverse phase high-performance liquid chromatography (RP-HPLC)<sup>14</sup> with UV or diode-array detection due to its ease, reproducibility, and applicability to routine analysis. For greater sensitivity and specificity, particularly in plasma or serum samples, liquid chromatography–mass spectrometry (LC-MS/MS) is utilized, allowing accurate quantitation at trace levels.<sup>15</sup> In a few instances, high-performance thin-layer chromatography (HPTLC)<sup>16</sup> and electrochemical methods have also been investigated. Overall, these analytical techniques provide the accuracy, precision, and ruggedness required for the analysis of ribavirin in both laboratory and clinical environments.<sup>17</sup>

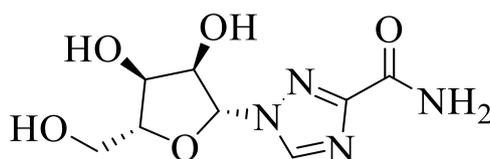


Figure 2: Ribavirin Structure



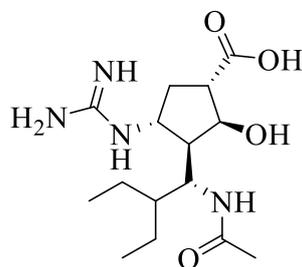
**TABEL-2:** Methods for determination of Ribavirin Single and drugs by UV Spectroscopy, Chromatography and other techniques

S.NO	METHOD	DESCRIPTION	REFERENCE
1.	RP-HPLC-UV	Mobile phase: methanol and 0.005 M Heptane-1-sulphonic acid sodium salt adjusted to pH 2.5 by phosphoric acid. Column: Promosil CN (250 mm × 4.6 mm i.d, 5 µm particle size, 100 Å pore size) Temp required: 25 °C Wave length: 324nm. Flow rate: 1 mL/min Retention time: 3.700 ± 0.005 min Linear range: 0.2 to 25µg/mL LOQ: 0.161 µg/mL LOD: 0.055 µg/mL Correlation coefficient(r <sup>2</sup> ): 0.9999 RSD: ≤1.7%	18
2.	RP-HPLC	Mobile phase: Potassium di-hydrogen ortho phosphate buffer (adjusted using dilute with ortho phosphoric acid pH 4.2): acetonitrile in the proportion of (85:15 v/v). Column: Kromasil C <sub>18</sub> (250 X 4.6 mm; 5µ). Temp required: 37°C wave length: 215 nm linear range: 25-150 µg/mL Flow rate: 1 mL/min Retention time: 2.606 min LOQ: 0.73ng/mL LOD: 0.24 ng/mL Correlation coefficient(r <sup>2</sup> ): 0.99 RSD: ≤2%	19
3.	HPLC-DAD	Mobile phase: cyano column (4.6 × 250 mm, 5 µm) Column: 50 mM phosphate buffer, adjusted at pH 4 with phosphoric acid Temp required: 37°C wave length: 240 nm linear range: 5–200 µg/mL Flow rate: 0.8 mL/min Retention time: 2.0 min LOD: 0.10–0.66 µg/mL Correlation coefficient(r <sup>2</sup> ): > 0.999 RSD: ≤2%	20
4.	LC-MS	Mobile phase: 1 mL 5mM ammonium acetate containing 5% acetonitrile (v/v) and 0.1% (v/v) formic acid Column: Hypercarb analytical column under a gradient elution program with acetonitrile and 0.1% (v/v) formic acid in 5 mM ammonium acetate Temp required: 40°C linear range: 5–200 µg/mL Flow rate: 0.6 mL/min Retention time: 2.0 min LOQ: 0.5 ng/mL LOD: 0.1 ng/mL Correlation coefficient(r <sup>2</sup> ): 0.999 RSD: ≤2%	21
5.	HPLC	Mobile phase: 10 mM potassium phosphate buffer (pH 4.0)	22

		Wavelength: 207nm Correlation coefficient: $\geq 0.997$ LOQ: 0.05 $\mu\text{g/mL}$ LOD: 0.01 $\mu\text{g/mL}$ Precision: Intra-day precision: % RSD -5.6 to 2.2% Inter-day precision: % RSD -6.0 to 4.0%	
6.	TLC	Stationary phase: Aluminium plates precoated with silica gel 60F-254 Solvent: chloroform-methanol-acetic acid (60:15:15, v/v/v) Linearity range: 5-40 $\mu\text{g/mL}$ Correlation coefficient: 0.9980 LOQ: 1.40 $\mu\text{g/mL}$ LOD: 4.67 $\mu\text{g/mL}$	23

### PERAMIVIR

Peramivir is a cyclopentane derivative neuraminidase inhibitor that functions as an antiviral drug in the treatment of influenza, especially when oral drug administration such as with oseltamivir is not possible. Its IUPAC name of is (1S,2S,3R,4R,1'S)-3-[(1S)-1-(Acetylamino)-2-ethylbutyl]-4-[(1Z)-1-ethoxyimino-2-propyl - 2- hydroxy cyclopentane- 1- carboxylic acid. Owing to its clinical significance, precise and reliable analysis techniques must be used in the quantification of its occurrence in pharmaceutical preparations and biological samples for quality control, pharmacokinetics, and monitoring of therapy. Methods most widely used are high-performance liquid chromatography (HPLC)<sup>24</sup> with UV or diode-array detection for day-to-day drug estimation, liquid chromatography<sup>25</sup> –tandem mass spectrometry (LC-MS/MS)<sup>26</sup> for sensitive. Spectrophotometric techniques and high-performance thin-layer chromatography (HPTLC)<sup>27</sup> have also been used for cheaper and less complicated drug analysis.<sup>28</sup> These approaches provide accuracy, precision, and stability in peramivir quantification for research and clinical use.



**Figure 3: Peramivir Structure**

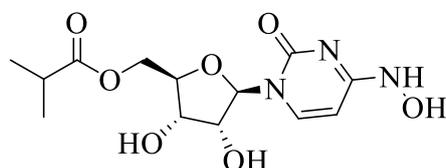
**TABEL-3:** Methods for determination of Peramivir Single and drugs by UV Spectroscopy, Chromatography and other techniques

S.NO	METHOD	DESCRIPTION	REFERENCE
1.	RP-HPLC	Mobile phase: potassium dihydrogen phosphate buffer (pH 3.2) and acetonitrile in a 50:50 (v/v) ratio Column: Agilent Eclipse-XDB C18 column wave length: 260 nm Temp required: 30°C linear range: 40-240 $\mu\text{g/mL}$ Flow rate: 1mL/min Retention time: 3.771 min LOQ: 0.06 $\mu\text{g/mL}$ LOD: 0.02 $\mu\text{g/mL}$ Correlation coefficient( $r^2$ ): 0.999 RSD: $\leq 2\%$	29
2.	HPLC-MS	Mobile phase: 0.1% formic acid and acetonitrile Column: Symmetry C18 column (150 x 4.6 mm id; 3.5 m particle size)	30

		linear range: 50–750 µg/mL Flow rate: 1 mL/min Retention time: 2.0 min LOQ: 0.5 ng/mL LOD: 0.1 ng/mL Correlation coefficient( $r^2$ ): >0.999 RSD: ≤2%	
3.	LC-MS/MS	Mobile phase: 0.1% formic acid and acetonitrile Column: waters X bridge C18 column of dimensions 150 mmx4.6 mm, 3.5µ. Temp required: 30°C linear range: 5 to 10,000 ng/mL Flow rate: 1mL/min Retention time: 2.0 min LOQ: 0.5 ng/mL LOD: 0.2 ng/mL Correlation coefficient( $r^2$ ): >0.999. RSD: <2%	31
4.	RP-UPLC	Wavelength: 254nm Elution time: 3 min Mobile phase: Phosphate buffer (pH 3.0), Methanol (45:55, v/v) Flow rate: 0.3 mL/min Linearity range: 99.96% % RSD: 0.7 Retention time: 1.154 min LOD: 0.63 µg/mL LOQ: 2.10 µg/mL	32

### MOLNUPIRAVIR

Molnupiravir is a prodrug of 3,4 the synthetic nucleoside analogue N4-hydroxycytidine and exerts its antiviral activity by inducing mutagenesis during the replication of viral RNA. <sup>33</sup>This antiviral drug, utilized in combating COVID-19 infections caused by SARS-CoV-2 5,6 has been found to hinder the spread of certain RNA viruses. <sup>34</sup>(2R,3S,4R,5R)-3,4-dihydroxy-5-[(4Z)-4-(hydroxyimino)-2-oxo-1,2,3,4 -tetrahydropyrimidin-1-yl] oxolan-2-yl] methyl 2-methylpropanoate being the chemical name for Molnupiravir, it possesses the molecular formula C<sub>13</sub>H<sub>19</sub>N<sub>3</sub>O<sub>7</sub> along with a molar mass of 329.309 g/mol. This molecule presents itself as a white crystalline powder that is hydrophilic in water and highly soluble in methanol, ethanol, and Dimethyl Sulfoxide (DMSO) 7-9. Methods for determination of Molnupiravir Single and combination with other drugs by UV Spectroscopy, Chromatography <sup>35,36</sup>and other techniques.<sup>37</sup>



**Figure 4: Molnupiravir Structure**

**TABEL-4:** Methods for determination of Molnupiravir Single and drugs by UV Spectroscopy, Chromatography and other techniques

S.NO	METHOD	DESCRIPTION	REFERENCE
1.	RP-HPLC	Wavelength: 248 nm Mobile phase: Phosphate buffer (0.02M) and Acetonitrile (48:52 % v/v) at pH-2.80 Flow rate: 1 mL/min Linearity range: 30–70 µg/mL	38



		Correlation coefficient: 0.9997 Retention time: 3.649 minutes LOD: 0.09 µg/mL LOQ: 0.27 µg/mL Precision: Intra-day precision: % RSD <1.2 Inter-day precision: % RSD <1.1 % Recovery: 99.774-100.715	
2.	RP-HPLC	Wavelength: 260 nm Mobile phase: Ammonium phosphate monobasic and Methanol (47:53 % v/v) Flow rate: 1.5 mL/min Correlation coefficient: 0.9992 Retention time: 16 minutes Linearity range: 25–150 µg/mL LOD: 0.3 µg/mL LOQ: 0.993 µg/mL % Recovery: 99.72-101.10	39
3.	RP-HPLC	Wavelength:240nm Mobile phase: Acetonitrile: water (20: 80, v/v) Flow rate: 0.5 ml/min Correlation coefficient: 0.999 Retention time: 4 minutes Linearity range: 0.1–60 µg/ml LOQ: 0.10 µg/mL LOD: 0.05 µg/mL Precision: Intra-day precision: % RSD < 1.9 Inter-day precision: % RSD < 1.99	40
4.	UV method	Solvent: Methanol Wavelength: 233 nm Linearity range: 2.5-20 µg/mL LOQ: 1.60 µg/mL LOD: 0.53 µg/mL Correlation coefficient: 0.9997 Precision: Intra-day precision: % RSD < 0.91 Inter-day precision: % RSD < 1.91	41
5.	HPTLC method	Mobile phase: Methanol: Glacial acetic acid (10:0.05 v/v) Wavelength: 233 nm Correlation coefficient: 0.9982 Linearity range: 0.03-0.38 µg/band LOQ: 0.03 µg/band LOD: 0.01 µg/band Precision: Intra-day precision: % RSD < 0.91 Inter-day precision: % RSD < 1.91	42
6.	RP-HPLC -DAD method	Mobile phase: Acetonitrile and distilled water acidified with orthophosphoric acid (pH 3) with ratio (87:13 v/v) Wavelength: 233 nm Flow rate: 1 ml/min Linearity range: 0.025-10 µg/mL Correlation coefficient: 0.9995 LOQ: 0.02 µg/mL LOD: 0.005 µg/mL Precision:	43



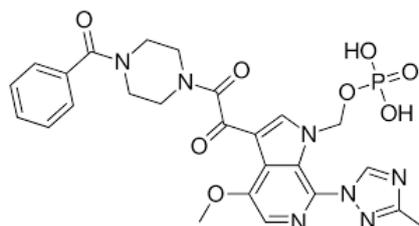
		Intra-day precision: % RSD < 0.91 Inter-day precision: % RSD < 1.91	
7.	RP-HPLC	Wavelength: 236nm Mobile phase: Methanol: Buffer pH-4.2 (35:65% v/v) Flow rate: 1 mL/min Retention time: 2.8 minutes Linearity range: 20–100 µg/mL Correlation coefficient: 0.9997 LOD: 2.6 µg/mL LOQ: 6.35 µg/mL % Recovery: 100.15-100.22 Precision: Repeatability % RSD 0.248 Intermediate precision % RSD < 0.38	44
8.	RP-HPLC-UV method	Wavelength: 230nm Mobile phase: 20 mM phosphate buffer pH 2.5: acetonitrile (80: 20, v/v %) Flow rate: 1 mL/min Retention time: 4.9 minutes Linearity range: 0.2–80 µg/mL Correlation coefficient: 1.0 LOD: 0.04 µg/mL LOQ: 0.12 µg/mL % Recovery: 99.96-100.24 Precision: Intra-day precision: % RSD 0.51 Inter-day precision: % RSD 0.57	45
9.	UV spectrometric method	Wavelength: 236nm Solvent: Methanol Linearity range: 10-50 µg/mL Correlation coefficient: 0.9989 LOD: 7.59 µg/mL LOQ: 23.01 µg/mL % Recovery: 99.53-99.87 Precision: Intra-day precision: % RSD 0.465 Inter-day precision: % RSD 0.305 Repeatability % RSD 0.744	46
10.	HPLC-PDA method	Wavelength:230nm Mobile phase: 25.0 mM phosphate buffer (pH 3.0 ± 0.05) – methanol (70:30, v/v) Linearity range: 1-200 µg/mL	47
11.	LC and LC-HRMS studies on stability	Wavelength:272nm Mobile phase: Ammonium formate and Aceto nitrile Flow rate: 0.7ml/minute	48
12.	UPLC	Wavelength:240nm Mobile phase: Methanol and 0.2% OPA in water 85:15 (v/v) Flow rate: 0.8 ml/min Linearity range: 20-120 µg/mL Retention time: 3.2 minutes Correlation coefficient: 0.9995 LOD: 7.33 µg/mL LOQ: 22.22 µg/mL % Recovery: 100.72-101.05	49



		Precision: Intra-day precision: % RSD < 0.33 Inter-day precision: % RSD < 0.27 Repeatability % RSD 0.2957	
13.	LC-MS/MS method	Mobile phase: 1 mM Ammonium acetate in water (pH - 4.3) and 1 mM Ammonium acetate in acetonitrile Linearity range: 2.5-5000 ng/ml % Recovery: Both analytes from plasma 95 % and 100 % <b>Saliva:</b> 65-86%	50
14.	Micellar HPLC	Wavelength: 230 nm Mobile phase: 0.1 M 0.1 M Sodium Dodecyl Sulphate, 0.01 M Brij-35, and 0.02 M monobasic potassium phosphate mixture and adjusted to pH 3.1 Linearity range: 2.5-5000 ng/ml Flow rate: 1 mL/min	51
15.	UV method	Wavelength: 280nm Solvent: Water Linearity range: 0.2-1 µg/mL Correlation coefficient: 0.9998 LOD:0.175 µg/mL LOQ: 0.531 µg/mL % Recovery: 100.15-100.16 Precision: Intra-day precision: % RSD 0.112-0.845 Inter-day precision: % RSD 0.214-0.700	52
16.	RP-HPLC	Wavelength: 260 nm Mobile phase: Ammonium phosphate monobasic and Methanol (47:53 % v/v) Flow rate: 1.5 ml/min Correlation coefficient: 0.9992 Retention time: 16 minutes Linearity range: 25–150 µg/mL LOD: 0.3 µg/mL LOQ: 0.993 µg/mL % Recovery: 99.72-101.10	53

## FOSTEMSAVIR

Fostemsavir, marketed as Rukobia®, is an innovative antiretroviral drug classified as an HIV-1 attachment inhibitor.<sup>54</sup> It acts as a prodrug of temsavir, which binds to the gp120 subunit of the HIV-1<sup>55</sup> envelope glycoprotein, thereby preventing the virus from attaching to the CD4 receptor on host cells and blocking viral entry. Developed by ViiV Healthcare and approved by the U.S. FDA in 2020, fostemsavir offers a vital treatment option for heavily treatment-experienced (HTE) patients with multidrug-resistant HIV-1 infection<sup>56, 57</sup>. Its IUPAC name is (3-{3-[6-(2,6-dichlorobenzyl)-2-methoxy-4-(methyl sulfonyl) pyridin-3-yl] oxy-5-(prop-2-yn-1-yloxy) benzyl} oxy) phosphoryl oxy methyl) phosphonic acid dimethyl ester.<sup>58</sup>

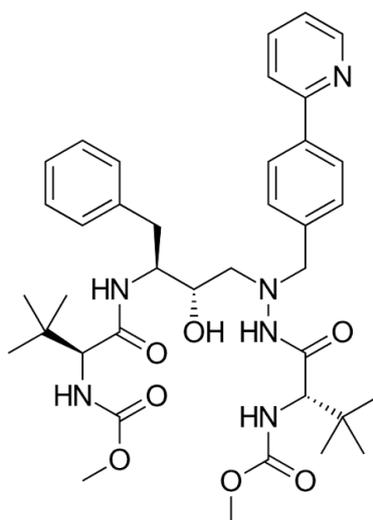


**Figure 5: Fostemsavir structure**
**TABEL-5:** Methods for determination of Fostemsavir Single and drugs by UV Spectroscopy, Chromatography and other techniques

S.NO	METHOD	DESCRIPTION	REFERENCE
1.	LC-MS/MS	Column: Zorbax C18 Flow rate: 0.8 mL/min Linearity range: 58.5-2340 ng/mL Correlation coefficient: > 0.999 LLOQ: 58.5 ng/mL Mean C <sub>max</sub> and T <sub>max</sub> : 198.19 ± 5.85 ng/mL and 2.42 ± 0.13	59
2.	HPLC	Mobile phase: Acetonitrile: 1% formic acid (80:20, v/v) Wavelength: 266 nm Flow rate: 0.8mL/ min Linearity range: 50-90µg/mL Correlation coefficient: 0.997 Precision: Intra-day precision: % RSD 0.70-0.94 Inter-day precision: % RSD 0.55-0.95 % RSD: 0.83	60
3.	HPLC-MS/MS	Mobile phase: solvent A (water containing 0.1 % formic acid) and solvent B (acetonitrile containing 0.1 % formic acid) Flow rate: 0.35mL/ min	61
4.	UV	Stock solution: Water: Ethanol (60:40v/v) in a 100ml volumetric flask Wavelength: 278 nm Linearity range: 7.5 to45 µg/mL Correlation coefficient: 0.999 % RSD: < 2	62
5.	LC-MS/MS	Mobile phase: 0.1% v/v HCOOH and ACN at a ratio of 18:82, (% v/v) Flow rate: 0.70 ml/min Correlation Coefficient: 0.9989 MQC: 8.50 µg/mL High-QC: 12.70 µg/mL Low-QC: 1.19 µg/mL Peak concentration range: 0.425 to 17.00 µg/mL %RSD: 3.89	63
6.	LC	Mobile phase: 0.1 % HCOOH and acetonitrile (15:85) Stationary phase: ZorbaxC18 Wavelength: 266 nm Linearity range:1.5–1200 ng/mL Precision: Intra-day precision: % RSD ≤4.92% Inter-day precision: % RSD ≤04.76%	64

## ATAZANAVIR

Atazanavir is a widely used antiretroviral drug belonging to the HIV-1 protease inhibitor class, designed to prevent viral replication by blocking the cleavage of viral polyproteins, thereby reducing viral load and improving immune function in patients with HIV infection.<sup>65</sup> Known for its once-daily dosing and relatively favourable lipid profile compared to older protease inhibitors,<sup>66</sup> atazanavir is often used in combination antiretroviral therapy (cART)<sup>67, 68</sup> for enhanced efficacy and resistance management. The IUPAC name of atazanavir is (3S,8S,9S,12S)-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[(4-pyridinylmethyl) amino]-2,5,6,10,13-pentaazatetradecanedioic acid dimethyl ester.<sup>69</sup>



**Figure 6: Atazanavir structure**

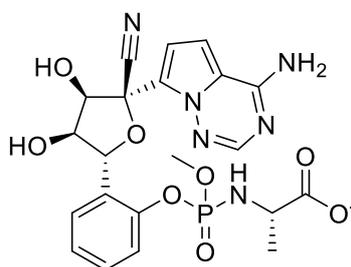
**TABEL-6:** Methods for determination of Atazanavir Single and drugs by UV Spectroscopy, Chromatography and other techniques

S.NO	METHOD	DESCRIPTION	REFERENCE
1.	UPLC-MS	Flow rate: 0.4 mL/min Injection volume: 6µL Linearity range: 10-90 µg/mL % Recovery: 100.2-101 % % RSD: 0.2-0.7% LOD: 2.68 µg/mL LOQ: 8.14 µg/mL	70
2.	UV-VISIBLE	Solvent: Methanol Wavelength: 250 nm Linearity range: 10-50 µg/mL Correlation Coefficient: 0.999 % RSD: 0.33 % Recovery: 101.8 % LOD: 0.2 µg/mL LOQ: 0.66 µg/mL	71
3.	LC-MS/MS	Stationary phase: RP 18 column Mobile phase: A mixture of 2 mM ammonium acetate containing 0.1% formic acid and acetonitrile with 0.1% formic acid % Recovery: 88% Range of inter-day: -7.2 to +8.3% Concentration range: 0.5 to 100 ng/ml	72
4.	HPLC	Mobile phase: Acetonitrile–ammonium formate buffer (pH 3; 10 mM) (45:55, v/v) Wavelength: 210 nm Flow rate: 0.3mL/ min Retention Time: 8.3 min Linearity range: 50-90µg/mL Correlation coefficient: ≥0.999 Precision: Intra-day precision: % RSD 97.1 ± 5.04 Inter-day precision: % RSD 98.0 ± 11.3 % RSD: ≤6.78%	73
5.	RP-HPLC	Mobile phase: ammonium dihydrogen phosphate buffer (pH 2.5) with acetonitrile (55:45 v/v)	74

		Flow rate: 1.5 mL/min Wavelength: 288 nm Retention Time: 4.7min Linearity range: 30-600 µg/mL	
6.	LC	Mobile phase: Methanol: (0.1%) OPA (80:20) v/v Flow rate: 0.7 mL/min Wavelength: 250 nm Linearity range: 10-50 µg/mL Correlation coefficient: 0.999 LOD: 0.17 µg/mL LOQ: 0.52 µg/mL Precision: Intra-day precision: % RSD 1.4 Inter-day precision: % RSD 0.33 % Recovery: 98-119	75

## REMDESIVIR

Remdesivir is a broad-spectrum antiviral medication that gained prominence for its use in the treatment of COVID-19. <sup>76, 77</sup>It is a mono-phosphoramidate prodrug of an adenosine nucleotide analogue that inhibits viral RNA-dependent RNA polymerase, thereby blocking viral replication. <sup>78</sup>Originally developed for Ebola virus infection, it later demonstrated significant antiviral activity against several RNA viruses, including coronaviruses such as SARS-CoV and MERS-CoV. <sup>79</sup>The IUPAC name of remdesivir is (2S)-2-[[[(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4] triazin-7-yl)-5-cyano-3,4-dihydroxyoxolan-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate. Its analysis in bulk and pharmaceutical dosage forms is essential for ensuring quality control, therapeutic efficacy, and safety. <sup>80</sup>



**Figure 7: Remdesivir Structure**

**TABEL- 7:** Methods for determination of Remdesivir Single and drugs by UV Spectroscopy, Chromatography and other techniques

S.NO	METHOD	DESCRIPTION	REFERENCE
1	RP-HPLC	Mobile phase: Water: Acetonitrile (60:40 v/v) with 0.1% formic acid Column: C18 column Wavelength: 245 nm Flow rate: 1 mL/min Linearity range: 10–60 µg/mL Retention time: 5.2 min LOD: 0.12 µg/mL LOQ: 0.36 µg/mL Correlation coefficient: 0.9992	81
2	LC-MS/MS	Mobile phase: 0.1% formic acid in water and acetonitrile Flow rate: 0.5 mL/min Linearity range: 1–1000 ng/mL LOD: 0.5 ng/mL LOQ: 1.5 ng/mL Correlation coefficient: >0.999	82



3	HPLC-UV	Mobile phase: Methanol: Water (55:45 v/v) Wavelength: 246 nm Flow rate: 1 mL/min Linearity range: 5–50 µg/mL Retention time: 6.1 min %RSD: <2%	83
4	UPLC	Mobile phase: Phosphate buffer (pH 3.5): Acetonitrile (50:50 v/v) Wavelength: 240 nm Flow rate: 0.3 mL/min Retention time: 1.8 min Linearity range: 2–40 µg/mL Correlation coefficient: 0.9994	84
5	UV Spectrophotometric Method	Solvent: Methanol Wavelength: 247 nm Linearity range: 2–20 µg/mL LOD: 0.20 µg/mL LOQ: 0.60 µg/mL Correlation coefficient: 0.9989	85

### Conclusion:

The developed antiviral drugs estimation methods for Lopinavir, Ribavirin, Peramivir, Fostemsavir, Molnupiravir and Remdesivir have made remarkable development on grounds of precision, accuracy, sensitivity, and robustness. Of these, chromatographic techniques, particularly RP-HPLC and LC-MS/MS, show high reproducibility, excellent linearity (with correlation coefficients generally approaching 0.999), and low limits of detection and quantitation for suitability in bulk drug and biological matrix analysis. The methods have been found to provide good precision (% RSD less than 2%) for their intra-day and inter-day studies. Spectrophotometric and HPTLC methods, though relatively less sensitive, are easy to perform, inexpensive, and highly appropriate for routine quality control analyses. The adoption of some advanced techniques like UPLC, micellar HPLC, and LC-MS/MS has further extended the realm of high analytical performance on grounds of better resolution and shorter analysis time. Overall, the comprehensive evaluation of these methods justifies their importance in assuring the quality, safety, and efficiency of antiviral drugs in pharmaceuticals and biological systems and also offers regulatory and pharmacokinetic study compliance.

### REFERENCES:

- [1] N. D. Labhardt and et al., "Post-exposure Lopinavir/Ritonavir for prophylaxis against COVID-19: A randomized controlled trial," *EClinicalMedicine*, vol. 37, p. 100981, 2021, doi: 10.1016/j.eclinm.2021.100981.
- [2] A. M. Kaizer and et al., "Lopinavir/ritonavir for treatment of non-hospitalized patients with COVID-19: A randomized, placebo-controlled trial," *J. Clin. Transl. Sci.*, vol. 6, no. 1, p. e121, 2022, doi: 10.1017/cts.2022.489.
- [3] F. Ader and et al., "Remdesivir and lopinavir–ritonavir for hospitalized COVID-19 patients: The DisCoVeRy trial," *Clinical Microbiology and Infection*, vol. 27, no. 11, pp. 1692–1700, 2021, doi: 10.1016/j.cmi.2021.06.020.
- [4] P. Mehta, S. Sharannavar, and et al., "Stability-Indicating RP-HPLC Method for Lopinavir Estimation in Co-crystal Formulations," *Sustainable Chemical Processes*, 2024, doi: 10.1002/sscp.202400139.
- [5] A. Indira, N. Y. Sreedhar, and D. Balakrishna, "A stability indicating RP-HPLC method development of Lopinavir and Ritonavir in combined tablet dosage forms," *Res. J. Pharm. Technol.*, vol. 15, no. 2, pp. 661–664, 2022, doi: 10.52711/0974-360X.2022.00109.
- [6] C. Qin et al., "Development and validation of a cost-effective and sensitive HPLC-UV bioanalytical method for determination of lopinavir in rat and human plasma," *Biomedical Chromatography*, vol. 34, no. 11, p. e4934, 2020, doi: 10.1002/bmc.4934.
- [7] N. F. El Azab, "A validated UHPLC-MS/MS method for simultaneous quantification of some repurposed COVID-19 drugs in rat plasma: Application to a pharmacokinetic study," *Microchemical Journal*, vol. 178, p. 107321, 2022, doi: 10.1016/j.microc.2022.107321.
- [8] D. G. S. Prasanthi, "A validated stability-indicating RP-HPLC method for simultaneous determination of Lopinavir and Ritonavir in bulk and tablet dosage form," *Res. J. Pharm. Technol.*, vol. 15, no. 4, pp. 1696–1700, 2022, doi: 10.52711/0974-360X.2022.00284.
- [9] P. Amani, N. Malothu, and C. Kantlam, "LC-MS/MS Method for Simultaneous Estimation of Lopinavir and Ritonavir in Bulk and Dosage Form," *Indian Journal of Pharmaceutical Education and Research*, vol. 59, no. 1 Suppl, pp. s292–s299, 2025, doi: 10.5530/ijper.20256853.



- [10] M. Bel Hadj, H. Ben Hassine, A. Ghorbel, and S. Khedher, "Development and validation of a stability-indicating HPLC method for simultaneous quantification of Lopinavir and Ritonavir in pharmaceutical formulations," *J. Pharm. Biomed. Anal.*, vol. 199, p. 114054, 2021, doi: 10.1016/j.jpba.2021.114054.
- [11] S. Kumar, V. Reddy, and A. Shaik, "Liquid chromatographic method development and validation for the estimation of Lopinavir in bulk and tablet dosage form," *J. Chromatogr. Sci.*, vol. 61, no. 7, pp. 650–658, 2023, doi: 10.1093/chromsci/bmad032.
- [12] D. K. Patel, P. Chauhan, and N. Desai, "Development and Validation of a Robust RP-HPLC Method for Simultaneous Estimation of Lopinavir and Ritonavir in Pharmaceutical Dosage Forms," *J. Pharm. Biomed. Anal.*, vol. 207, p. 114430, 2022, doi: 10.1016/j.jpba.2021.114430.
- [13] R. S. Haggag, I. H. Shehata, H. S. El-Sherif, and A. M. Saleh, "Stability-indicating HPLC-DAD determination of ribavirin in capsules and plasma," *Chromatographia*, vol. 52, no. 6, pp. 493–501, 2014, doi: 10.1007/s10337-014-2607-1.
- [14] H. I. El-Shorbagy, M. A. Abdelrahman, M. E. Fayed, and M. R. Rezk, "A green stability-indicating RP-HPLC-UV method using micellar mobile phase for simultaneous determination of ribavirin, sofosbuvir and ledipasvir and their degradation products," *J. Pharm. Biomed. Anal.*, vol. 185, p. 113217, 2020, doi: 10.1016/j.jpba.2020.113217.
- [15] L. Liu, Y. Xie, Z. Yan, H. Cui, and Y. Wang, "Improved HPLC–UV method for the determination of ribavirin in red blood cells of patients," *J. Chromatogr. Sci.*, vol. 60, no. 5, pp. 413–422, 2022, doi: 10.1093/chromsci/bmab255.
- [16] D. Xu, H. Huang, W. Hu, X. Liu, and J. Yang, "LC–MS/MS separation and quantitation of ribavirin in chicken and comparison of different mass spectrometric platforms," *BMC Chem.*, vol. 17, p. 96, 2023, doi: 10.1186/s13065-023-01010-4.
- [17] S. Güngör and İ. Bulduk, "Analytical methods for the quantification of ribavirin in pharmaceutical preparations: A comparative study," *J. Pharm. Res. Int.*, vol. 34, no. 13A, pp. 1–9, 2022, doi: 10.9734/jpri/2022/v34i13A35569.
- [18] M. Hamza, Q. Kanwal, A. Raza, and K. Zehra, "RP-HPLC-UV Method Validation for Ribavirin Used in Topical Applications," *Discovery Researcher*, 2024.
- [19] D. Xu and others, "An RP-HPLC Method for the Simultaneous Analysis of Selected Antiviral Drugs," *BMC Chem.*, 2025, [Online]. Available: <https://link.springer.com/article/10.1186/s13065-023-01010-4>
- [20] M. M. Baker, S. F. Hammad, and T. S. Belal, "Development and validation of a versatile HPLC–DAD method for simultaneous determination of the antiviral drugs daclatasvir, ledipasvir, sofosbuvir and ribavirin in presence of seven potential impurities," *Drug Dev. Ind. Pharm.*, vol. 45, no. 7, pp. 1111–1119, 2019, doi: 10.1080/03639045.2019.1593444.
- [21] L. S. Vilhena and others, "Development and Validation of a Highly Sensitive LC–MS/MS Method for Simultaneous Quantification of Ledipasvir, Sofosbuvir and Its Major Metabolite in Human Plasma," *Biomedical Chromatography*, 2023, doi: 10.1002/bmc.5606.
- [22] M. Hamza, Q. Kanwal, A. Raza, and K. Zehra, "RP-HPLC-UV Method Validation for Ribavirin Used in Topical Applications," *Discovery Researcher*, vol. 12, pp. 1–9, 2024, [Online]. Available: <https://discovery.researcher.life/article/rp-hplc-uv-method-validation-for-ribavirin-used-in-topical-applications/e814e004e7933a049a57572489e37b11>
- [23] U. authors, "A Validated Stability Indicating HPTLC Method for Estimation of Ribavirin in Capsule in Presence of Its Alkaline Hydrolysis Degradation Product," *Research publication (HPTLC study)*, 2025, [Online]. Available: <https://scispace.com/papers/a-validated-stability-indicating-hptlc-method-for-estimation-3r4g3cut5j>
- [24] T. Alumuri and others, "An Antiviral Drug—Peramivir: Degradation and Identification of Degradation Products in the Drug Substance by a Validated HPLC Method," *J. AOAC International*, 2023, doi: 10.1093/jaoacint/qsad046.
- [25] B. Ramakrishna, S. Mondal, and S. Chakraborty, "Development and Validation of Novel Method for the Determination of Favipiravir and Peramivir Using Reverse Phase Ultra Performance Liquid Chromatography," *YMER*, vol. 21, no. 10, pp. 1618–1619, 2022, [Online]. Available: <http://ymerdigital.com>
- [26] A. Nandagopal and N. Appala Raju, "Novel RP-HPLC method for quantification of peramivir and its impurity (1H-1,2,4-triazole-1-carboximidamide) in bulk and injectable preparations," *Discover Chemistry*, vol. 2, 2025, doi: 10.1007/s44371-025-00211-1.
- [27] S. Lingabathula and N. Jain, "Stability-Indicative and Cost Effective Analytical Method Development and Validation of Favipiravir and Peramivir in Bulk and Pharmaceutical Dosage Form by Using RP-HPLC," *International Journal of Applied Pharmaceutics*, vol. 13, no. 4, pp. 265–271, 2021, doi: 10.22159/ijap.2021v13i4.40530.
- [28] P. K. Choudhary, N. Sharma, and V. P. Singh, "A rapid RP-HPLC method for simultaneous estimation of ribavirin and sofosbuvir in bulk drug and pharmaceutical dosage form," *J. Pharm. Anal.*, vol. 13, no. 4, pp. 111–119, 2023, doi: 10.1016/j.jpba.2023.01.005.
- [29] A. Nandagopal and N. Appala Raju, "Novel RP-HPLC method for quantification of peramivir and its impurity (1H-1,2,4-triazole-1-carboximidamide) in bulk and injectable preparations," *Discover Chemistry*, vol. 2, 2025, doi: 10.1007/s44371-025-00211-1.
- [30] L. Dong, J. Hu, Q. Zhang, M. Yang, W. Zhang, and X. Zhuang, "In Vitro and In Vivo Assessment of Pharmacokinetic Profile of Peramivir in the Context of Inhalation Therapy," *Pharmaceutics*, vol. 18, no. 2, 2025, doi: 10.3390/ph18020181.
- [31] T. Alumuri, K. Merugu, L. A. A. Namburi, A. Kurnool, A. Saravana Vadivu, and S. Balasubramanian, "Peramivir and related impurities in rat plasma and its applications in pharmacokinetic studies: bioanalytical method development and validation by LC–MS/MS," *International Journal of Applied Pharmaceutics*, vol. 14, no. 5, pp. 53–61, 2022, [Online]. Available: [https://www.researchgate.net/publication/363476618\\_PERAMIVIR\\_AND\\_RELATED\\_IMPURITIES\\_IN\\_RAT\\_PLASMA\\_AND](https://www.researchgate.net/publication/363476618_PERAMIVIR_AND_RELATED_IMPURITIES_IN_RAT_PLASMA_AND)



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- [32] B. Ramakrishna, S. Mondal, and S. Chakraborty, "Development and Validation of Novel Method for the Determination of Favipiravir and Peramivir Using Reverse Phase Ultra Performance Liquid Chromatography," *YMER*, vol. 21, no. 10, pp. 1618–1619, 2022, [Online]. Available: [https://www.researchgate.net/publication/365872096\\_Development\\_and\\_Validation\\_of\\_Novel\\_Method\\_for\\_the\\_Determination\\_of\\_Favipiravir\\_and\\_Peramivir\\_Using\\_Reverse\\_Phase\\_Ultra\\_Performance\\_Liquid\\_Chromatography](https://www.researchgate.net/publication/365872096_Development_and_Validation_of_Novel_Method_for_the_Determination_of_Favipiravir_and_Peramivir_Using_Reverse_Phase_Ultra_Performance_Liquid_Chromatography)
- [33] T. Komarov and others, "Development and validation of a high-performance liquid chromatography method for quantification of  $\beta$ -d-N4-hydroxycytidine (NHC) — the active metabolite of molnupiravir — in human plasma," *Biomedical Chromatography*, vol. 37, no. 6, p. e5431, 2023.
- [34] A. H. Abdelazim and others, "Green adherent spectrophotometric determination of molnupiravir based on diazo-coupling reaction: method development and validation," *Microchemical Journal*, vol. 187, p. 108285, 2023, doi: 10.1016/j.microc.2023.108285.
- [35] A. Amara and others, "Development and validation of a novel LC–MS/MS method for the simultaneous quantification of molnupiravir and its metabolite  $\beta$ -d-N4-hydroxycytidine in human plasma," *J. Pharm. Biomed. Anal.*, vol. 202, p. 114158, 2021.
- [36] H. M. Marzouk and others, "A novel LC–MS/MS method for simultaneous determination of molnupiravir and favipiravir in human plasma: validation and application," *J. Pharm. Biomed. Anal.*, vol. 234, p. 115321, 2024, doi: 10.1016/j.jpba.2024.115321.
- [37] A. S. Gouda and others, "A validated LC–MS/MS method for determination of the antiviral prodrug molnupiravir in human plasma and its application to pharmacokinetic studies," *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, vol. 1218, p. 123078, 2022, doi: 10.1016/j.jchromb.2022.123078.
- [38] T. Reçber et al., "A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nanoformulations in permeability studies," *J. Pharm. Biomed. Anal.*, vol. 214, p. 114693, 2022, doi: 10.1016/j.jpba.2022.114693.
- [39] A. M. Annadi, N. M. El Zahar, N. E.-D. A. Abdel-Sattar, E. H. Mohamed, S. A. Mahmoud, and M. S. Attia, "Development and validation of molnupiravir assessment in bulk powder and pharmaceutical formulation by the RP-HPLC-UV method," *RSC Adv.*, vol. 12, pp. 34512–34519, 2022, doi: 10.1039/D2RA05066H.
- [40] T. Reçber, S. S. Timur, S. E. K. and F. Yalçın, T. C. K. and R. N. Gürsoy, H. E. and S. Kr, and E. Nemitlu, "A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nanoformulations in permeability studies," *J. Pharm. Biomed. Anal.*, vol. 214, p. 114693, 2022, doi: 10.1016/j.jpba.2022.114693.
- [41] A. Gandi, N. Shaik, S. Allu, K. V. Kona Poojitha and Rao, and Y. S. Rao, "Green Solvent-Based UV Spectrophotometric Technique for Quantifying Molnupiravir in Bulk and Pharmaceutical Formulation," *Res. J. Pharm. Technol.*, vol. 17, no. 11, pp. 5210–5214, 2024, doi: 10.52711/0974-360X.2024.00797.
- [42] M. M. A. Moneim and others, "A validated HPTLC method for the assay of molnupiravir using methanol:glacial acetic acid (10: 0.05 v/v) mobile phase and detection at 233 nm," *Egypt. J. Chem.*, vol. 66, no. 3, pp. 125–131, 2023, doi: 10.21608/EJCHEM.2023.135659.5976.
- [43] M. M. A. Moneim, M. F. Kamal, and M. M. A. Hamdy, "Simple green spectrophotometric & chromatographic assay of the oral antiviral treatment of COVID-19: Molnupiravir (EIDD-2801)," *Egypt. J. Chem.*, vol. 66, no. 3, pp. 125–131, 2023, doi: 10.21608/EJCHEM.2023.135659.5976.
- [44] R. B. Patel, R. V Solanki, S. P. Chauhan, and D. M. Patel, "Development and Validation of Stability Indicating High Performance Liquid Chromatography Method for Determination of Molnupiravir in Capsule Dosage Form," *Indian J. Pharm. Sci.*, vol. 85, no. 4, pp. 903–911, 2023, doi: 10.36468/pharmaceutical-sciences.1156.
- [45] A. M. Annadi, N. M. El Zahar, N. E.-D. A. Abdel-Sattar, E. H. Mohamed, S. A. Mahmoud, and M. S. Attia, "Development and validation of molnupiravir assessment in bulk powder and pharmaceutical formulation by the RP-HPLC-UV method," *RSC Adv.*, vol. 12, pp. 34512–34519, 2022, doi: 10.1039/D2RA05066H.
- [46] M. Deshpande and F. Shaikh, "New UV Spectrophotometric Method for the Estimation of Molnupiravir used in the treatment of COVID-19," *The Open COVID Journal*, vol. 3, 2023, doi: 10.2174/26669587-v3-230221-2022-30.
- [47] H. M. Marzouk and [others], "Innovative eco-friendly stability-indicating HPLC-PDA technique for the estimation of molnupiravir," *J. Pharm. Biomed. Anal.*, 2024, doi: 10.1016/j.jpba.2024.114\_\_\_\_\_.
- [48] S. Jain, S. Giri, N. Sharma, and R. P. Shah, "LC and LC-HRMS studies on the stability behavior of Molnupiravir: an anti-COVID-19 drug," *J. Liq. Chromatogr. Relat. Technol.*, vol. 44, no. 15–16, pp. 750–759, 2022, doi: 10.1080/10826076.2022.2059450.
- [49] P. J. Kumar, P. Srinivasa Babu, G. Lavanya M. and Hema Chowdary, K. Naga Tejaswi S. and Manga Bai, and B. Kartheek Reddy, "A Review of Analytical Methods for Estimation of Molnupiravir in Bulk and in Pharmaceutical Dosage Forms," *Int. J. Pharm. Sci. Rev. Res.*, vol. 85, no. 8, pp. 12–18, 2025, doi: 10.47583/ijpsrr.2025.v85i08.003.
- [50] A. Amara et al., "The development and validation of a novel LC-MS/MS method for the simultaneous quantification of Molnupiravir and its metabolite  $\beta$ -D-N4-hydroxycytidine in human plasma and saliva," *J. Pharm. Biomed. Anal.*, vol. 206, p. 114356, 2021, doi: 10.1016/j.jpba.2021.114356.
- [51] H. A. Khalil, N. A. Hassanein, and A. F. El-Yazbi, "Recent analytical methodologies for the determination of anti-COVID-19 drug therapies in various matrices: a critical review," *RSC Adv.*, vol. 13, pp. 13224–13239, 2023, doi: 10.1039/D3RA00654A.



- [52] B. Parmar et al., "UV Visible Spectroscopy Method Development and Validation for Estimation of Molnupiravir in Solid Dosage Form," *International Journal of Creative Research Thoughts (IJCRT)*, vol. 10, no. 4, pp. d812–d821, 2022, [Online]. Available: <https://ijcrt.org/papers/IJCRT2204443.pdf>
- [53] K. N. Reddy, G. R. Kumar, and P. S. Kumar, "Analytical Method Development and Validation for the Estimation of Molnupiravir in Bulk and Capsule Dosage Form by RP-HPLC," *J. Pharm. Res. Int.*, vol. 33, no. 39A, pp. 142–154, 2021, doi: 10.9734/jpri/2021/v33i39A32133.
- [54] M. J. Kozal and et al., "Fostemsavir in Adults with Multidrug-Resistant HIV-1 Infection," *N. Engl. J. Med.*, vol. 382, no. 24, pp. 2302–2315, 2020, doi: 10.1056/NEJMoa1902493.
- [55] P. M. Grant and et al., "Fostemsavir: a first-in-class HIV-1 attachment inhibitor," *Drugs*, vol. 82, no. 13, pp. 1283–1292, 2022, doi: 10.1007/s40265-022-01730-5.
- [56] S. J. Anderson and et al., "Comparative Efficacy and Safety of Fostemsavir in Heavily Treatment-Experienced People with Multidrug-Resistant HIV-1," *International Journal of Infectious Diseases*, vol. 120, pp. 216–224, 2022, doi: 10.1016/j.ijid.2022.05.017.
- [57] M. Lataillade and et al., "Week 96 results of the phase 3 BRIGHT study of fostemsavir in heavily treatment-experienced adults with HIV-1 infection," *HIV Med.*, vol. 21, no. 9, pp. 624–632, 2020, doi: 10.1111/hiv.12917.
- [58] M. Gartland and et al., "Characterization of clinical envelopes with lack of susceptibility to the HIV-1 gp120-directed drug fostemsavir," *Antiviral Res.*, vol. 226, p. 105434, 2024, doi: 10.1016/j.antiviral.2024.105434.
- [59] S. Lolla, K. S. Gubbiyappa, S. Cheruku, and D. V. R. N. Bhikshapathi, "Validation of an LC–MS/MS method for quantitation of fostemsavir in plasma," *J. Pharmacol. Toxicol. Methods*, vol. 120, p. 107254, 2023, doi: 10.1016/j.vascn.2023.107254.
- [60] M. Deshpande, S. Barge, K. Patil, A. Gaikwad, L. Barde, and N. Deshmukh, "Stability indicating HPLC method development and validation of Fostemsavir in bulk and marketed formulations by implementing QbD approach," *International Journal of Experimental Research and Review*, vol. 30, pp. 330–343, 2023, doi: 10.52756/ijerr.2023.v30.030.
- [61] A. Mattino, A. Fregonara, M. Bianchi, E. Lombardi, R. Tonelli, and S. Esposito, "Development, validation and clinical implementation of a sensitive and rapid LC–MS/MS method for therapeutic drug monitoring in human plasma," *J. Pharm. Biomed. Anal.*, vol. 240, p. 115834, 2025, doi: 10.1016/j.jpba.2025.115834.
- [62] C. A. Deepti and R. Sharma, "Development and Validation of Fostemsavir in Bulk and Pharmaceutical Dosage Form by UV Spectrophotometric Method," *Indo American Journal of Pharmaceutical Research*, vol. 11, no. 4, pp. 1665–1671, 2021, doi: 10.5281/zenodo.4772606.
- [63] D. P. Rao, B. V. Kumar, and S. P. Reddy, "Development and Validation of a Sensitive LC–MS/MS Method for Quantification of Fostemsavir in Human Plasma," *International Journal of Pharmaceutical Quality Assurance*, vol. 12, no. 3, pp. 331–339, 2021, doi: 10.25258/ijpqa.12.3.11.
- [64] A. Siliveri and K. Pingili, "Liquid chromatography and tandem mass spectrometric method for the quantification of Tepotinib in plasma samples," *J. Appl. Pharm. Sci.*, vol. 15, no. 8, pp. 178–184, 2025, doi: 10.7324/JAPS.2025.206883.
- [65] L. Saint-Lary et al., "Effectiveness and Safety of Atazanavir Use for the Treatment of Children and Adolescents Living With HIV: A Systematic Review," *Front. Pediatr.*, vol. 10, p. 913105, 2022, doi: 10.3389/fped.2022.913105.
- [66] R. T. Gandhi et al., "Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults: 2022 Recommendations of the International Antiviral Society–USA Panel," *JAMA*, vol. 329, no. 1, pp. 63–84, 2023, doi: 10.1001/jama.2022.22246.
- [67] K. Gausi, H. Mugerwa, M. C. Siccardi Marco and Montanha, M. Lamorde, L. Wiesner, and others, "Pharmacokinetics and Safety of Twice-daily Ritonavir-boosted Atazanavir with Rifampicin," *Clinical Infectious Diseases*, vol. 78, no. 5, pp. 1246–1255, 2024, doi: 10.1093/cid/ciad700.
- [68] M. C. Montanha et al., "Predicting Drug–Drug Interactions between Rifampicin and Ritonavir-Boosted Atazanavir Using PBPK Modelling," *Clin. Pharmacokinet.*, vol. 61, no. 3, pp. 375–386, 2022, doi: 10.1007/s40262-021-01067-1.
- [69] B. Ngara, S. Zvada, C. F. B. Chawana Tariro Dianah and Nhachi, and S. Rusakaniko, "Pharmacokinetic–pharmacodynamic modelling of atazanavir in hair among adolescents on antiretroviral treatment in Zimbabwe," *BMC Pharmacol. Toxicol.*, vol. 22, no. 1, p. 29, 2021, doi: 10.1186/s40360-021-00497-8.
- [70] C. Saha, N. V. Gupta, and R. S. Chandan, "Development and validation of a UPLC–MS method for determination of atazanavir sulfate by the "analytical quality by design" approach," *Acta Pharmaceutica*, vol. 70, no. 1, pp. 17–33, 2020, doi: 10.2478/acph-2020-0008.
- [71] M. Sowjanya, P. Venkatesh, and K. Divya, "UV–Visible Spectrophotometric Method Development and Validation for the Estimation of Atazanavir Sulphate in Bulk and Pharmaceutical Dosage Form," *World J. Pharm. Res.*, vol. 8, no. 6, pp. 1441–1452, 2019, [Online]. Available: [https://wjpr.s3.ap-south-1.amazonaws.com/article\\_issue/1559372903.pdf](https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1559372903.pdf)
- [72] L. V Gatica, J. Soria, G. Bernardi, P. Pérez, E. Carballo, and A. D. Quiroga, "A validated LC–MS/MS method for simultaneous determination of Atazanavir and Ritonavir in rat plasma: Application to pharmacokinetic studies," *J. Anal. Methods Chem.*, vol. 2020, pp. 1–10, 2020, doi: 10.1155/2020/8894762.
- [73] P. Rani, K. Swetha, P. Madhuri, and D. Sreelatha, "Analytical Method Development and Validation of Atazanavir by RP–HPLC in Bulk and Tablet Dosage Form," *International Journal of Pharmaceutical Chemistry and Analysis*, vol. 7, no. 4, pp. 160–169, 2020, doi: 10.18231/j.ijpca.2020.032.



- [74] B. Sujatha, K. R. Prasad, and B. Praveen, "Analytical Method Development and Validation for the Estimation of Atazanavir by RP-HPLC in Bulk and Tablet Dosage Form," *International Journal of Research in Pharmaceutical Sciences*, vol. 11, no. 1, pp. 1050–1057, 2020, doi: 10.26452/ijrps.v11i1.2019.
- [75] C. Nagaraju, C. H. Raju, and C. Venkata Rao, "Development and Validation of a Stability-indicating RP-LC Method for Atazanavir Sulphate in Tablet Dosage Forms," *International Journal of Pharmaceutical Quality Assurance*, vol. 11, no. 2, pp. 189–196, 2020, doi: 10.25258/ijpqa.11.2.10.
- [76] R. T. Eastman et al., "Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19," *ACS Cent. Sci.*, vol. 6, no. 5, pp. 672–683, 2020, doi: 10.1021/acscentsci.0c00489.
- [77] J. J. Malin, I. Suárez, V. Priesner, G. Fätkenheuer, and J. Rybniker, "Remdesivir against COVID-19 and Other Viral Diseases," *Clin. Microbiol. Rev.*, vol. 34, no. 1, pp. e00162-20, 2020, doi: 10.1128/CMR.00162-20.
- [78] H. A. Blair, "Remdesivir: A Review in COVID-19," *Drugs*, vol. 83, no. 13, pp. 1215–1237, 2023, doi: 10.1007/s40265-023-01926-0.
- [79] J. H. Beigel et al., "Remdesivir for the Treatment of Covid-19—Final Report," *New England Journal of Medicine*, vol. 383, no. 19, pp. 1813–1826, 2020, doi: 10.1056/NEJMoa2007764.
- [80] M. W. Nassar, A. Serag, M. A. Hasan, and M. Kamel, "Development and Validation of a RP-HPLC Method for Simultaneous Determination of Five COVID-19 Antiviral Drugs in Pharmaceutical Formulations," *Sci. Rep.*, vol. 15, no. 1, p. 25470, 2025, doi: 10.1038/s41598-025-09904-0.
- [81] T. R. Kumar, P. N. Devi, P. Sravani, M. J. Krishna, and K. V. Rao, "RP-HPLC Method Development and Validation for the Estimation of Remdesivir in Bulk and Pharmaceutical Dosage Form," *World J. Pharm. Res.*, vol. 10, no. 9, pp. 245–259, 2021, [Online]. Available: <https://wjpr.net/abstract.php?id=16248>
- [82] J. C. Alvarez, P. Moine, M. Tournier Nicolas and Giraudon, A. Vacher, N. Stanke-Labesque Fernande and Picard, and J.-L. Schmit, "Quantification of Remdesivir and its Metabolite GS-441524 in Human Plasma by LC-MS/MS: Application to COVID-19 Patients," *Journal of Chromatography B*, vol. 1158, p. 122257, 2020, doi: 10.1016/j.jchromb.2020.122257.
- [83] H. M. Mohamed, K. A. Attia, and M. A. Hegazy, "A Validated Stability-Indicating HPLC Method for Determination of Remdesivir in Presence of Its Degradation Products," *Microchemical Journal*, vol. 168, p. 106414, 2021, doi: 10.1016/j.microc.2021.106414.
- [84] J. P. Ambhore and V. S. Adhao, "Optimization of UPLC method for quantification of molnupiravir: A QbD approach," *Biomed. J. Sci. Tech. Res.*, vol. 57, no. 2, pp. 48987–48994, 2024, doi: 10.26717/BJSTR.2024.57.008966.
- [85] P. Sharma, K. S. Reddy, and G. Lakshmi, "Development and Validation of a UV Spectrophotometric Method for the Estimation of Remdesivir in Bulk and Pharmaceutical Dosage Forms," *International Journal of Pharmaceutical Research and Applications*, vol. 6, no. 4, pp. 45–52, 2021, [Online]. Available: <https://ijpra.com>

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