Review Article

A Review of Analytical Methods for Estimation of Molnupiravir in Bulk and in Pharmaceutical Dosage Forms

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ABSTRACT

The estimation of Molnupiravir in bulk and dosage forms is essential for quality control and regulatory compliance in pharmaceutical manufacturing. Various analytical techniques, including UV spectroscopy, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), liquid chromatography-mass spectrometry (LC-MS), and high-performance thin-layer chromatography (HPTLC), have been developed and validated for this purpose. These methods enable accurate and precise qualitative and quantitative determination of Molnupiravir and its related impurities or degradants. Their application ensures the reliability of pharmaceutical formulations and supports the optimization of drug development and quality assurance processes.

Keywords: Molnupiravir, analysis, RP-HPLC, UV, LC-MS

INTRODUCTION

The global COVID-19 pandemic, caused by SARS-CoV-2, has resulted in nearly 270 million confirmed cases and over 5.2 million reported fatalities[1] worldwide. Despite these staggering figures, efforts to identify more effective therapeutic options to combat COVID-19 persist. Among these, antiviral candidates hold significant promise as potential treatments, particularly for high-risk [2] patient populations.

Molnupiravir, a prodrug of [3,4] the synthetic nucleoside analogue N4-hydroxycytidine, manifests its antiviral efficacy by inducing mutagenesis during the replication of viral RNA. This antiviral agent, employed in combating COVID-19 infections caused by SARS-CoV-2 [5,6] impedes the propagation of specific RNA viruses. Chemically designated as [(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 (Figure 1), Molnupiravir exhibits the molecular formula $C_{13}H_{19}N_3O_7$ and a molar mass of 329.309 g/mol. This compound appears as a white crystalline substance, displaying hydrophilicity in water and notable solubility in methanol, ethanol, and dimethyl sulfoxide (DMSO)[7-9]. Methods for determination of Molnupiravir Single and combination with other drugs by UV Spectroscopy, Chromatography and other techniques shown in the table 1.

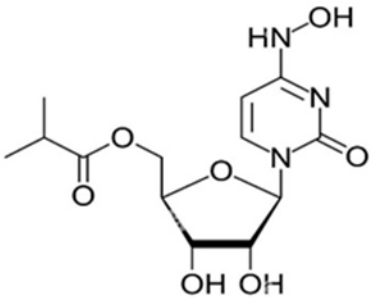

Figure 1: Molnupiravir structure

Table 1: Methods for determination of Molnupiravir Single and combination with other drugs by UV Spectroscopy, Chromatography and other techniques

Sr. No	Drug	Method	Description	RFR
1	Molnupiravir in API form and marketed pharmaceutical dosage form	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 **Correlation coefficient** : 0.9997 **Retention time** : 3.649 minutes **LOD** : 0.09 µg/ml **LOQ** : 0.27 µg/m **Precision :** **Repeatability**: % RSD : 0.200979 **Intra-day precision** : % RSD <1.2 **inter-day precision** : % RSD <1.1 **% Recovery** : 99.774-100.715	10

2	Molnupiravir in API	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	11
3	Molnupiravir in nano formulations	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	12
4	Molnupiravir in capsule Dosage form	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 **Accuracy and intra-day precision % RSD** : 99.92-101.75, < 0.91 **Accuracy and inter-day precision % RSD** : 100.20-101.55, < 1.91	13
		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 **Accuracy and intra-day precision % RSD** : 99.92-101.75, < 0.91 **Accuracy and inter-day precision % RSD**: 100.20-101.55,< 1.91	
		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 **Accuracy and intra-day precision % RSD** : 99.92-101.75, < 0.91 **Accuracy and inter-day precision % RSD** : 100.20-101.55, < 1.91	
5	Molnupiravir in API & tablet dosage form	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	14

			LOD : 2.6µg/ml **LOQ** : 6.35µg/m **% Recovery** : 100.15-100.22 **Precision** : **Repetability % RSD** : 0.248 **Intermediate precision % RSD**: < 0.38	
6	Molnupiravir in Dosage form	RP-HPLC	**Wavelength** : 234nm **Mobile phase** : water : Acetonitrile (70:30 % v/v) **Flow rate** : 1 ml/min **Retention time**: 3.5 minutes **Linearity range** : 10–50 µg/ml **Correlation coefficient:** 0.9991 **LOD** : 4.058µg/ml **LOQ** : 13.50µg/m **% Recovery** : 99.30-101.49 **Precision** : **Intra-Day Precision % RSD** : 0.187 **Inter-Day Precision % RSD** : 0.227 **Repetability % RSD** : 0.203	15
7	Molnupiravir in Pure bulk powder and Capsule dosage form	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/m **% Recovery** : 99.96-100.24 **Precision**: **Intraday precision RSD %** : 0.51 **Interday precision RSD %** : 0.57	16
8	Molnupiravir in bulk and pharmaceutical dosage form	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	17
9	Molnupiravir in bulk and formulation	UV spectrometric method	**Wavelength** : 235nm **Solvent** : Water **Linearity range** : 2-10 µg/ml **Correlation coefficient** : 0.9999 **LOD** : 1 µg/ml **LOQ** : 1.5 µg/ml **% Recovery** : 99.16-99.95 **Precision** : **Intra-Day Precision % RSD**: < 1.84 **Inter-Day Precision % RSD**: < 1.69 **Repeatability % RSD**: < 0.16	18
10	Molnupiravir in	RP-HPLC method	**Wavelength** : 236nm **Mobile Phase** : Methanol: CAN : Water (50:20:30v/v/v) pH-3.1 with orthophosphoric acid **Linearity range** : 10-50 µg/ml **Retention time** : 2.427 minutes **Correlation coefficient :** 0.9991	19

			LOD : 1.3376 µg/ml **LOQ** : 4.0536 µg/ml **% Recovery** : 98.00-103.31 **Precision** : **Intra-Day Precision % RSD : 1.295** **Inter-Day Precision % RSD** : 1.3	
11	For simultaneous determination of COVID-19 infection molnupiravir and favipiravir	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	20
12	Molnupiravir in stability behaviour	LC and LC-HRMS studies on stability	**Wavelength** : 272nm **Mobile Phase**: Ammonium formate and Aceto nitrile **Flow rate:** 0.7ml/minute	21
13	Estimation of Molnupiravir	UV method RP-HPLC method	**Wavelength** : 235nm **Linearity range** : 4-9 g/ml **Correlation coefficient** : 0.999 **Wavelength** : 235nm **Mobile Phase** : Ethanol and phosphate buffer (42.5:57.5% v/v) **flow rate** : 0.9 mL/min **Linearity range** : 10-150 g/ml **Correlation coefficient** : 0.999	22
14	Molnupiravir in Human plasma	RP-HPLC method	**Mobile phase** : Methanol: acidified water adjusted at pH 4.0 with 0.1 M orthophosphoric acid; with ratio (70:30, v:v) **flow rate** : 0.6 mL/min **Wavelength** : 240 nm **Linearity range** : **Molnupiravir** : 4–120 µg/mL **Dexamethasone** : 10–140µg/mL **Ertapenem** : 10–160 µg/mL **Remdesivir** : 4–120 µg/mL	23
15	Molnupiravir in Pharmaceutical formulations	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 **Precision** : **Intraday precision RSD %:** < 0.33 **Interday precision RSD %** : < 0.27 **Repetability RSD % :** 0.2957	24
16	Molnupiravir in Bulk & Tablet dosage form	RP-HPLC method	**Wavelength** : 270nm **Mobile phase**: Methanol and Phosphate Buffer was in the ratio of 25:75%v/v (pH-3.4) **Flow rate** : 1 ml/min **Linearity range** : 5-15 µg/ml **Retention time** : 2.784 minutes **Correlation coefficient :** 0.9996 **LOD** : 0.6902 µg/ml **LOQ** : 2.091 µg/ml **% Recovery** : 99.83-100.780 **Precision**: **Intraday precision RSD %** : 0.3 **Interday precision RSD%** : 0.258 **Repeatability RSD %** : 0.36	25

17	Molnupiravir in Capsule Dosage Form	RP-HPLC method	**Wavelength** : 270nm **Mobile phase** : buffer (pH 4.5) and methanol (70:30 v/v), **Flow rate** : 1 ml/min **Linearity range** : 49.80-149.40 µg/ml **Retention time** : 2.784 minutes **Correlation coefficient** : 0.999 **LOD** : 0.25 µg/ml **LOQ** : 0.5 µg/ml **% Recovery** : 99.19-100.73 **Precision** : **Method precision RSD %** : 1.23 **Interday precision RSD %** : 1.01 **System Precision RSD %** : 0.11	26
18	Molnupiravir in pure blend and its dosage form.	RP-HPLC method	**Wavelength** : 235nm **Mobile phase** : Water: acetonitrile (70: 30 % v/v) **Flow rate** : 1 ml/min **Linearity range** : 10-50 µg/ml **Retention time** : 4.009 minutes **Correlation coefficient :** 0.9997 **LOD** : 0.1 µg/ml **LOQ** : 0.32 µg/ml **% Recovery** : 98.40-100.62 **Precision** : **Intraday precision RSD %** : <1.65 **Interday precision RSD %** : <1.16	27
19	Molnupiravir in Bulk and capsule	RP-HPLC method	**Wavelength** : 246nm **Mobile phase** : Phosphate Buffer: acetonitrile (60: 40 % v/v) pH=2.8 **Flow rate** : 1 ml/min **Linearity range** : 30-70 µg/ml **Retention time** : 3.668 minutes **Correlation coefficient :** 0.9997 **LOD** : 3.19 µg/ml **LOQ** : 9.69 µg/ml **% Recovery** : 99.74-100.477 **Precision**: **Intraday precision RSD %** : 0.31 **Interday precision RSD %** : 0.19 **Retability RSD %** : 0.200	28
20	Molnupiravir in Pharmaceutical dosage form	RP-HPLC method	**Wavelength** : 254 nm **Mobile phase** : Orthophosphoric acid: Acetonitrile 60:40 % v/v **Flow rate** : 1 ml/min **Linearity range** : 10-60 µg/ml **Retention time** : 2.46 minutes **Correlation coefficient :** 0.9997 **LOD** : 0.06 µg/ml **LOQ** : 0.21 µg/ml **% Recovery** : 98.71-101.86 **Precision** : **Intermediate precision RSD %** : 0.9	29
21	Molnupiravir and its metabolite ß-d-N4-hydroxycytidine in human plasma and saliva	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 % **Saliva** : 65-86%	30

22	Determination of Molnupiravir and Favipiravir	Micellar HPLC	**Wavelength** : 230 nm **Mobile phase** : 0.1 M 0.1 M Sodium Dodecyl Sulfate , 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	31
23	Molnupiravir in solid dosage form	UV method	**Wavelength** : 280nm **Solvent** : Water **Linearity range** : 0.2-1 µg/ml **Correlation coefficient** : 0.9998 **LOD** : 0.175µg/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	32
24	Molnupiravir in API	UV method	**Wavelength** : 280nm **Solvent** : Water **Linearity range** : 5-30 µg/ml **Correlation coefficient** : 0.999 **LOD** : 1.1µg/ml **LOQ** : 3.3 µg/ml **% Recovery** : 99.15-99.97 **Precision** : **Intra-Day Precision % RSD** : 0.112-0.845 **Inter-Day Precision % RSD** : 0.214-0.700	33

CONCLUSION

The analytical methods for determining Molnupiravir in various matrices, including API, pharmaceutical dosage forms, and biological samples, demonstrate significant advancements in precision, accuracy, and sensitivity. Among the most common methods, RP-HPLC exhibits versatility with varied mobile phases, wavelengths, and flow rates, ensuring applicability across formulations. Correlation coefficients consistently near or equal to 0.999 indicate robust linearity, while low LOD and LOQ values highlight the methods' sensitivity, especially in formulations like human plasma and bulk drugs. Precision metrics, with RSD values below 2%, ensure reliability for intra-day and inter-day analyses. UV spectrophotometry serves as a simpler, cost-effective alternative for routine analysis, albeit with slightly lower sensitivity compared to chromatographic techniques. The incorporation of advanced methods like LC-MS/MS and micellar HPLC further supports the quantification of Molnupiravir and its metabolites in complex matrices, ensuring comprehensive analysis. Collectively, these methods provide reliable, reproducible tools for quality control and pharmacokinetic studies of Molnupiravir.

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