Ind. J. Pharm. Edu. Res., 2027; 1-6.
https://www.ijper.org

Original Article

Method Development and Validation of Assay of Molnupiravir in Molnupiravir Capsules by RP-HPLC

Sumit Agarwal[1,*], Rajasekaran Sidhan[2]

[1]Department of Pharmaceutics, Bhagwant University, Sikar, Ajmer, Rajasthan, INDIA.
[2]Department of Pharmacology, Bhagwant University, Sikar, Ajmer, Rajasthan, INDIA.

ABSTRACT

Background: Method was developed and validated for performing Assay of Molnupiravir in Molnupiravir Capsules **Materials and Methods:** RP-HPLC with an auto sampler and 15 cm x 4.6 mm, 5 µm L1 (C18) column (Make: Intek Chromasol, Model: Chromasol Jade C18). 0.1% ortho phosphoric acid buffer, acetonitrile and methanol in ratio 80: 16: 04% was used as mobile phase and HPLC grade water was used as diluent. Flow rate and injection volume were 0.8 mL/min and 5 µL respectively. Detection wave length was 237 nm. **Results:** The method was found to be specific without any interference from blank, placebo, mobile phase solution and impurities. % RSD of six samples was 1.01 indicating the precision of the method. Accuracy and linearity of the method were determined over the concentration range of 50 to 150%. The mean % recovery was found to be 99.66%, Correlation coefficient (R^2) was found to be 0.9999. The method remained unaffected by the small changes in analytical method parameters like variation in flow rate±0.2 mL/min, temperature±2°C and wavelength±2 nm indicating the robustness of the method. **Conclusion:** The method was novel and simple with a run time of 10 min, hence can be utilized in industries for Assay of Molnupiravir in Molnupiravir Capsules.

Keywords: Assay, COVID-19, Molnupiravir Capsules, RP-HPLC.

Correspondence:
Mr. Sumit Agarwal
Research Scholar, Department of Pharmaceutics, Bhagwant University, Sikar, Ajmer, Rajasthan, INDIA.
Email: sumit@saingroup.com

Received: 27-05-2025;
Revised: 19-12-2025;
Accepted: 28-09-2026.

www.ijper.org

INTRODUCTION

Molnupiravir is an oral antiviral medication used to treat COVID-19 in individuals infected with SARS-CoV-2.[1–5] It is a prodrug that is metabolized into N4-hydroxycytidine, a synthetic nucleoside derivative. Chemical structure of Molnupiravir was provided in Figure 1. Once activated, Molnupiravir interferes with the replication of the virus by causing errors in the viral RNA. This leads to the formation of defective viral genomes, which impairs the virus's ability to reproduce. As a result, the viral load is reduced, helping to manage the infection effectively.[6–8] The current paper discusses the method development and validation of Assay method for quantitative estimation of Molnupiravir in Molnupiravir Capsules by RP-HPLC.[9,10]

DOI: 10.5530/ijper.20272890

Copyright Information :
Copyright Author (s) 2027 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

MATERIALS AND METHODS

Chemicals and Reagents

Molnupiravir API, Molnupiravir capsules- 200 mg, Ortho phosphoric acid, Methanol, Acetonitrile, HPLC grade Water.

Instrumentation

Analytical Weighing Balance, Sonicator, HPLC (With Auto Sampler)- Prominence - i LC-2030C Plus.

Chromatographic Conditions

Column: 15 cm x 4.6 mm, 5 µm L1 (C18) (Make: Intek Chromasol, Model: Chromasol Jade C18).

Flow Rate: 0.8 mL/min **Wave Length:** 237 nm **Injection Volume:** 5 µL **Temperature:** 40°C **Run Time:** 10 min.

Diluent: HPLC grade Water

Preparation of Buffer Solution

Accurately transferred 1 mL of Ortho phosphoric acid into 1-L HPLC grade water, shaken and mixed well. Filtered through 0.45 µ filter, sonicated to degas.

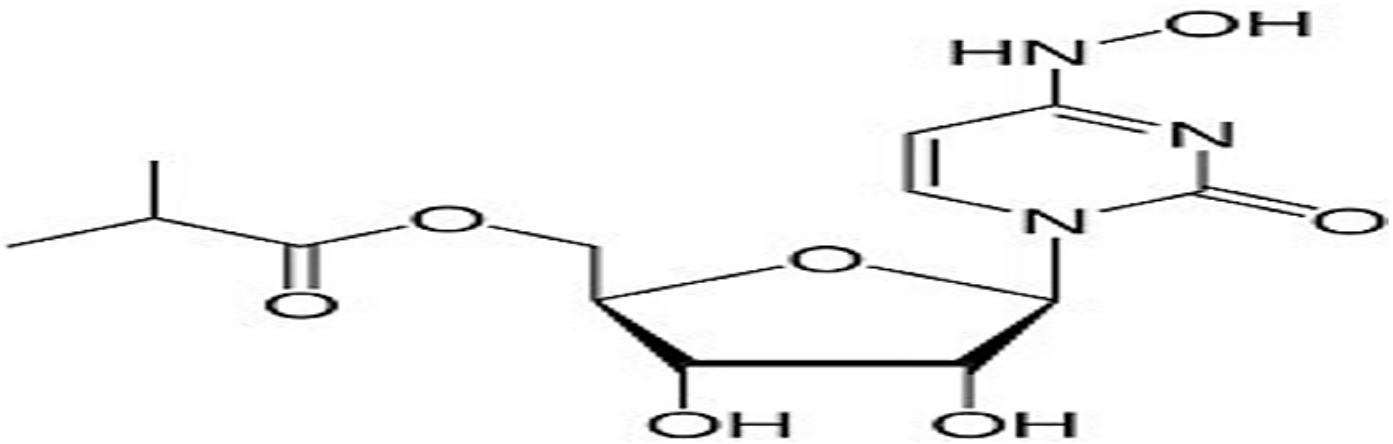


Figure 1: Chemical Structure of Molnupiravir.

Preparation of Mobile Phase

Buffer solution: Acetonitrile: Methanol (80: 16: 04%).

Preparation of Standard Stock Solution

Weighed and transferred 25 mg Molnupiravir working standard into 50 mL volumetric flask, added 20 mL of diluent and sonicated to dissolve, volume was made up to mark with diluent.

Preparation of Standard Solution

Diluted 10 mL of standard stock solution to 50 mL with diluent.

Preparation of Sample Stock Solution

Dissolved a weighed quantity of mixed contents of 20 capsules containing equivalent to about 25 mg of Molnupiravir in 50 mL volumetric flask by adding 20 mL of diluent and sonicating. Volume was made up to mark with diluent, mixed well and filtered.

Preparation of Sample Solution

Diluted 10 mL of filtered sample stock solution to 50 mL with diluent.

Spiked Sample Preparation

Diluted 10 mL of sample stock solution and 10 mL of standard stock solution to 50 mL with diluent.

Placebo Stock Preparation

Weighed accurately equivalent to 25 mg of placebo in 50 mL volumetric flask, added 20 mL diluent and shaken with aid of ultrasound for 15 min and diluted to volume with diluent and filtered.

Table 1: Results of System Suitability of Molnupiravir.

Parameter	Observed value
Tailing factor	1.099
Plate count	75314
Retention time	6.87
Parameter	Observed value
%RSD (peak areas)	0.09

Placebo Preparation

Diluted 10 mL of filtered placebo stock solution to 50 mL with diluent.

Method validation

The method developed was validated as per ICH Q2(R[1]) guidelines to check System Suitability, Specificity, Linearity, Accuracy, Precision, Robustness.[7]

RESULTS

System Suitability

The % RSD for peak areas was 0.09 for five replicate injections of Molnupiravir Standard, tailing factor was 1.099, Plate count was 75314. From the results of Table 1 it was concluded that system was meeting the System Suitability acceptance criteria.

Specificity

No interference from blank, mobile phase, placebo and impurities peaks was observed at the retention time of Molnupiravir peak which was evident from the results of Table 2. Thus specificity of the method was conformed (Figures 2-7).

www.ijper.org

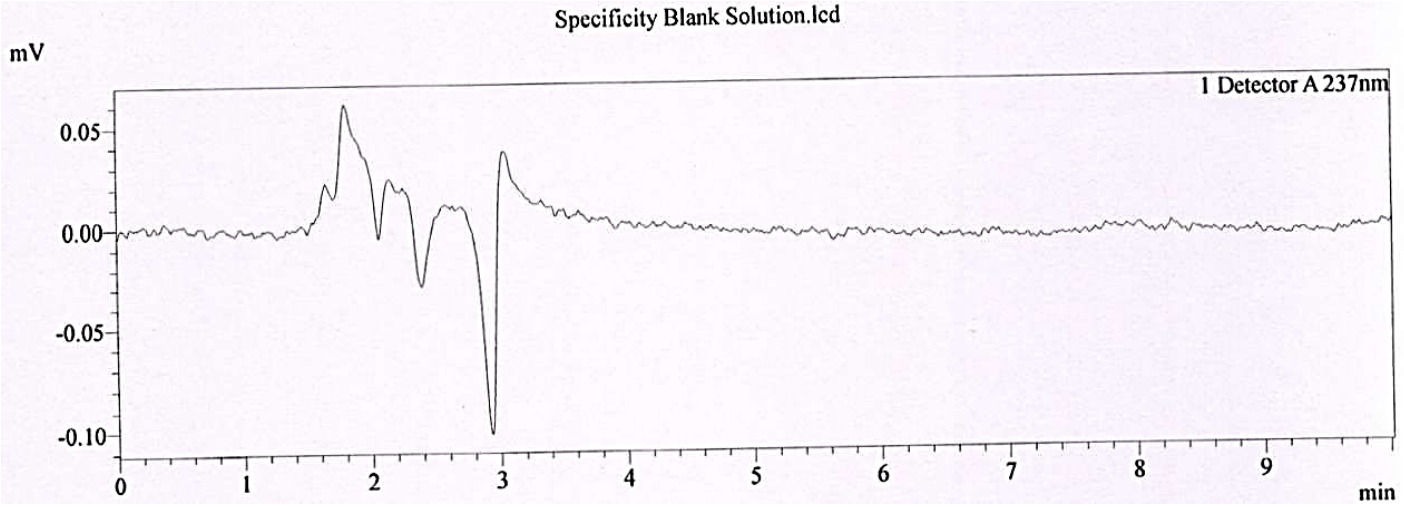
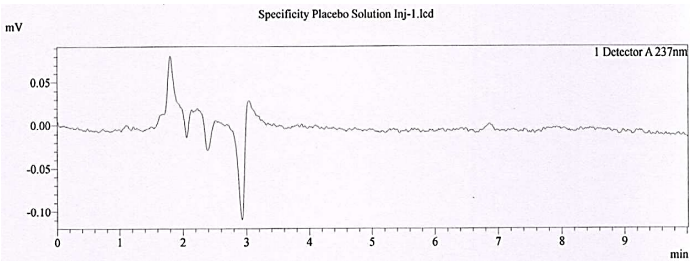


Figure 2: Chromatogram of Blank.

Table 2: **Results of Specificity of Molnupiravir.**

Sample Name	Inference
Blank	No interference was observed from Blank at retention time of Molnupiravir Peak.
Mobile Phase Solution	No interference was observed from Mobile Phase Solution at retention time of Molnupiravir Peak.
Placebo	No interference was observed from Placebo at retention time of Molnupiravir Peak.
Impurities	No interference was observed from Impurities peaks at retention time of Molnupiravir Peak.



Chromatogram of Spiked Sample.

Linearity

The method was found to be linear over the concentration of 50% to 150% with a Correlation Coefficient (R^2) 0.9999 (Figure 8 and Table 3).

Table 3: **Results of Linearity of Molnupiravir.**

Concentration Level of Molnupiravir	Peak Areas of Molnupiravir
50%	671304
75%	996737
100%	1321453
125%	1640390
150%	1983903
Concentration Level of Molnupiravir	**Peak Areas of Molnupiravir**
Correlation coefficient (R2) of Molnupiravir	0.9999

Accuracy

The Recovery values were found between 99.31% to 99.89% over the three concentration levels with a mean % Recovery of 99.66% and % RSD 0.21 conforming the Accuracy of method (Table 4).

Method Precision

The % RSD of six sample preparations of Molnupiravir Capsules was found to be 1.01 and Mean % Assay was 100.03 conforming the precision of the method which was evident from Table 5.

Robustness

Robustness of the method was investigated by varying analytical method parameters like flow rate ±0.2 mL/min, temperature ±2°C and wavelength ±2 nm. From the results of Table 6 the method was found to be robust.

DISCUSSION

The method was validated as per ICH Q2 (R^1) guidelines, demonstrating excellent specificity, precision, accuracy and

www.ijper.org

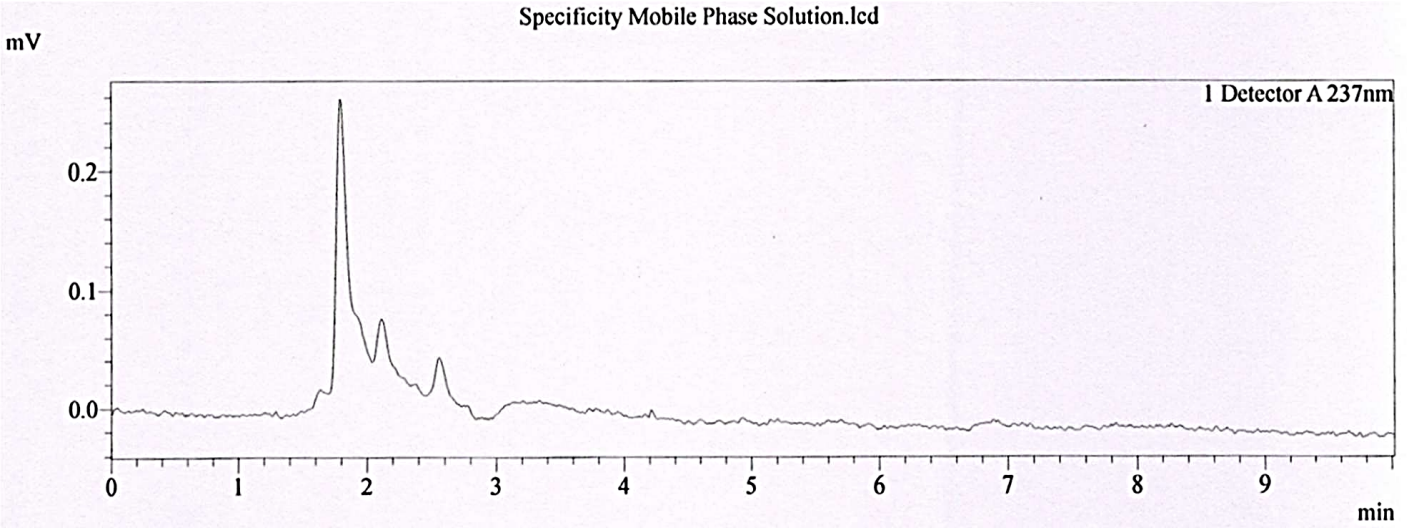


Figure 3

IMAGE MISSING

Figure 4: Chromatogram of Placebo.

Table 4: Results of Accuracy of Molnupiravir.

% Level	% Recovered
50	99.89
	99.73
	99.57
100	99.31
	99.72
	99.38
150	99.82
	99.65
	99.87
Mean % Recovery	99.66
% RSD	0.21

Table 5: Results of Method Precision of Molnupiravir.

Sample Number	% Assay of Molnupiravir
1	100.04
2	100.56
3	99.80
4	101.47
5	98.41
6	99.88
Mean	100.03
% RSD	1.01

Table 6: Results of Robustness of Molnupiravir.

	Lower variation	Actual	Higher variation
	% Assay		
Flow	100.24	100.31	100.39
Temperature	100.47	100.42	100.48
Wave length	98.76	98.68	98.53

linearity across the concentration range of 50-150% which was evident from the Results

www.ijper.org

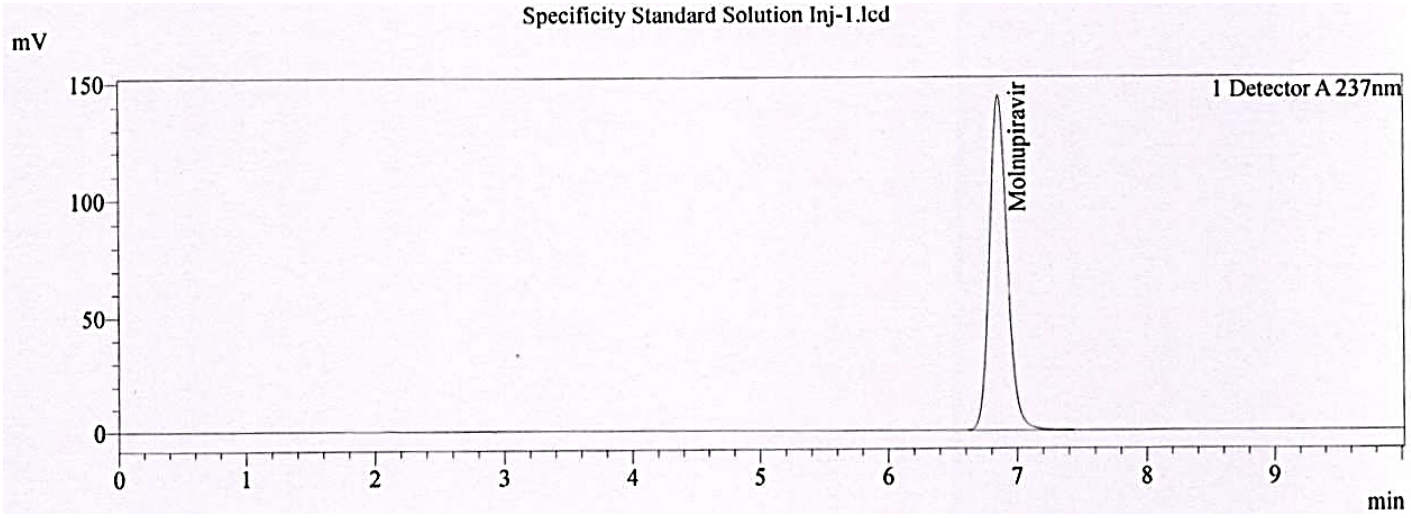


Figure 5: Chromatogram of Standard.

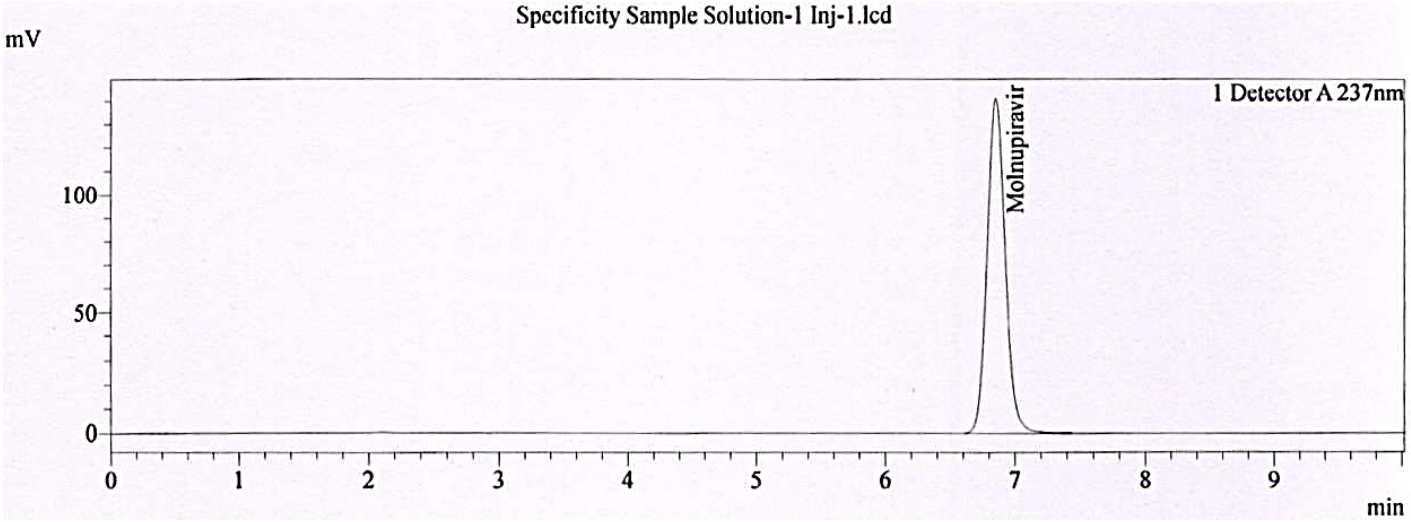


Figure 6: Chromatogram of Sample.

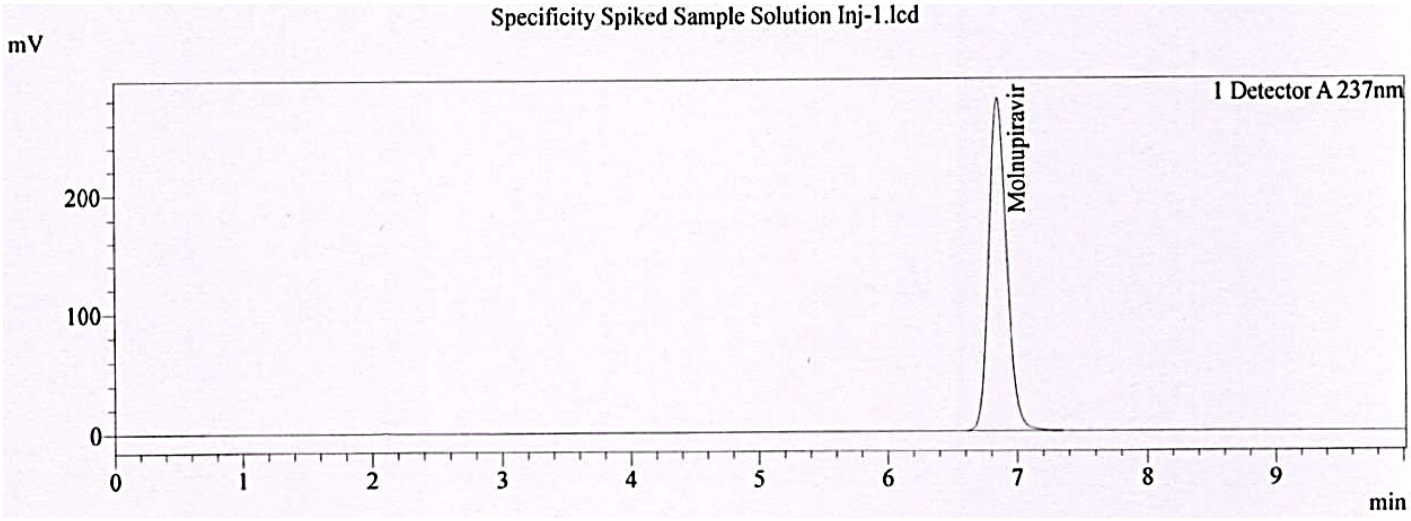


Figure 7

www.ijper.org

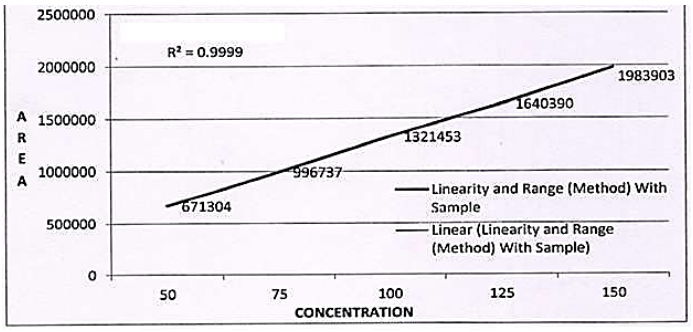


Figure 8: Linearity Graph of Molnupiravir.

CONCLUSION

A RP-HPLC method was developed and validated for Assay of Molnupiravir in Molnupiravir Capsules. The method was found to be specific without any interference from blank, placebo, mobile phase solution and impurities. % RSD of six samples was 1.01 indicating the precision of the method. Accuracy and linearity of the method were determined over the concentration range of 50 to 150%. Correlation coefficient (R^2) was found to be 0.9999. The method remained unaffected by the small changes in analytical method parameters like variation in flow rate ±0.2 mL/min, temperature ±2°C and wavelength ±2 nm indicating the robustness of the method. The Method was novel and simple with a run time of 10 min, hence can be utilized in industries for Assay of Molnupiravir in Molnupiravir Capsules.

ACKNOWLEDGEMENT

We express our sincere gratitude to Department of Pharmaceutics, Department of Pharmacology, Bhagwant University, Sikar Rd and Sain Medicaments Pvt. Ltd., Telangana for providing constant support, guidance and facilities to carry out the research work.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; **API:** Active Pharmaceutical Ingredient; **ICH:** International Council for Harmonisation; **RSD:** Relative Standard Deviation; **UV:** Ultraviolet; **Nm:** Nanometer; **µL:** Microliter; **mL:** Milliliter; **%RSD:** Percent Relative Standard Deviation; **HPLC:** High Performance Liquid Chromatography; **°C:** Degree Centigrade; **SARS-CoV-2:** Severe Acute Respiratory Syndrome Coronavirus 2; **COVID-19:** Coronavirus Disease 2019.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

We express our heartfelt thanks to Sain Medicaments Pvt. Ltd., Telangana for providing the facilities and required funding to carry out the research work.

SUMMARY

A Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Molnupiravir in capsule dosage form. The separation was achieved using a C18 column (15 cm × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1% orthophosphoric acid buffer, acetonitrile, and methanol in the ratio of 80:16:04 (%v/v). The method utilized a flow rate of 0.8 mL/min, detection at 237 nm, and an injection volume of 5 µL.

The method was validated as per ICH Q2 (R^1) guidelines, demonstrating excellent specificity, precision (%RSD=1.01), accuracy (mean recovery=99.66%), and linearity (R^2=0.9999) across the concentration range of 50-150%. The method was robust against small deliberate variations in flow rate, temperature, and detection wavelength. The total runtime of 10 min makes the method efficient and suitable for routine quality control analysis in the pharmaceutical industry.

REFERENCES

1. Tarole T, Daroi P, Munipalli VK, Warde S, Singh RS, Nandi A, Bhaskar V. Stability Indicating RP-HPLC Method Development for the Estimation of Molnupiravir in Capsule Dosage Form. *Int. J. Pharm. Sci. Drug Res*. 2023; 15(3):222-228.
2. Sravanthi G, Gandla KS, Repudi L. New analytical method development and validation for estimation of molnupiravir in bulk and tablet dosage form by RP-HPLC method. *Cellular, Molecular and Biomedical Reports* 2023; 3 (3): 130-136.
3. Abdelaziz M. Annadi, Noha M. El Zahar, Nour El-Din A. Abdel-Sattar, Ekram H. Mohamed, Safwat A. Mahmoud, Mohamed S. Attia. Development and validation of molnupiravir assessment in bulk powder and pharmaceutical formulation by the RP- HPLC-UV method. *RSC Advances* 2022; 12; 34512-34519.
4. Recber T. Timur S. S. Erdogan Kablan S. Yalcin F. Karabulut T. C. Neslihan Gursoy R. *et al*. A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nanoformulations in permeability studies. *Journal of Pharmaceutical and Biomedical Analysis* 2022; 214:114693.
5. Jain P, Bhamare M, Surana S. Quantitative estimation of molnupiravir by UV-Spectrophotometric method. *Int J Pharm Chem Anal* 2022; 9(1):35-39.
6. Goswami H, Alsumali A, Jiang Y, Schindler M, Duke ER, Cohen J, *et al*. Cost-effectiveness analysis of molnupiravir versus best supportive care for the treatment of outpatient COVID-19 in adults in the US. Pharmacoeconomics 2022; 40(7):699-714.
7. Bindu M, Gandla K, Vemireddy S, Samuel S, Praharsha Y. A validated stability indicating RP-HPLC method for the determination of molnupiravir in pharmaceutical dosage form. *World Journal of Advanced Research and Reviews* 2022; 15 (01): 580-590.
8. Jayk Bernal A, Gomes da Silva MM, Musungaie DB, Kovalchuk E, Gonzalez A, Delos Reyes V, *et al*. Molnupiravir for oral treatment of COVID-19 in nonhospitalized patients. *N Engl J Med* 2022; 386(6):509-20.
9. Rosenke K, Okumura A, Lewis MC, Feldmann F, Meade-White K, Bohler WF, *et al*. Molnupiravir inhibits SARS-CoV-2 variants including Omicron in the hamster model. *JCI Insight* 2022; 7(13):e160108.
10. Painter WP, Holman W, Bush JA, Almazedi F, Malik H, Eraut N, *et al*. Human safety, tolerability, and pharmacokinetics of molnupiravir, a novel broad-spectrum oral antiviral agent with activity against SARS-CoV-2. *Antimicrob Agents Chemother* 2021; 65(5). e02428-20.

Cite this article: Agarwal S, Sidhan R. Method Development and Validation of Assay of Molnupiravir in Molnupiravir Capsules by RP-HPLC. Indian J of Pharmaceutical Education and Research. 2027;1-6.

www.ijper.org