https://doi.org/10.46344/JBINO.2026.v15i01.21

TO STUDY PHARMACEUTICAL ANALYSIS ENHANCE THE SENSITIVITY OF THE UPLC METHOD ENABLING THE DETECTION AND QUANTIFICATION OF IMPURITIES

Usha. K.[1*] & Dr. K. Ram Prasad[2]

1*- Research Scholar, Faculty of Pharmaceutical Sciences, Motherhood University, Dehradun Road, Karoundi Village, Bhagwanpur Post, Roorkee Tehsil, Haridwar District, Uttarakhand State-247661.
2- Professor, Faculty of Pharmaceutical Sciences, Motherhood University, Dehradun Road, Karoundi Village, Bhagwanpur Post, Roorkee Tehsil, Haridwar District, Uttarakhand State-247661.

Email Id: ushapharma@gmail.com

ABSTRACT

The analysis of impurities in drug testing is of paramount importance. It serves to identify and manage harmful impurities, thereby ensuring the purity, stability, and safety of drugs while also guaranteeing their efficacy. Presently, a variety of techniques and methods are utilized for impurity analysis, which include chromatographic techniques such as ion chromatography, gas chromatography, and high-performance liquid chromatography, in addition to mass spectrometry techniques like liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry, as well as spectrophotometric methods such as UV-visible spectrophotometry. Nevertheless, impurity analysis techniques encounter several challenges, including the necessity to enhance sensitivity and accuracy, to overcome obstacles in analyzing complex sample impurities, and to tackle issues related to method reproducibility. The optimization of impurity analysis entails the introduction of advanced techniques such as ultra-high-performance liquid chromatography and chromatography-mass spectrometry, the establishment of standardized processes, the enhancement of personnel training, and the application of artificial intelligence and automation technology. These initiatives improve the levels of drug quality control, ensuring the provision of safe and effective medications.

INTRODUCTION

Sub-therapeutic drug exposure and impurity-related instability are well-recognized contributors to the development of antiviral resistance. Consequently, stringent pharmaceutical analysis is essential to ensure therapeutic effectiveness and long-term treatment success. Robust analytical control not only safeguards product quality but also supports consistent clinical performance throughout the product lifecycle (Andersson & Hughes, 2014). Accurate analytical evaluation ensures correct dosage strength, batch-to-batch consistency, reliable stability assessment, and effective detection and quantification of degradation products, as well as identification of process-related impurities (ICH, 2003; ICH, 2005). Even minor deviations in active pharmaceutical ingredient (API) concentration can compromise therapeutic efficacy and potentially promote resistance. Likewise, the presence of degradation products or residual impurities may alter drug performance and influence pharmacokinetic behavior. Advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), LC–MS, and validated stability-indicating methods play a critical role in maintaining drug quality, safety, and regulatory compliance. Particularly in the context of emerging viral infections, maintaining rigorous analytical control is fundamental to ensuring consistent antiviral performance. Antiviral resistance is not solely a virological concern; it is also closely linked to pharmaceutical quality. Inadequate impurity characterization and insufficient analytical oversight can compromise drug performance. Therefore, systematic impurity profiling and the implementation of validated analytical methodologies form the cornerstone of modern antiviral quality assurance.

In the pharmaceutical industry, chromatographic methods are widely employed for assay determination, purity evaluation, content uniformity testing, and analysis of dissolution samples (Kumar et al., 2020). These methods are required to be stability-indicating in nature, meaning they must be capable of effectively separating the active pharmaceutical ingredient (API) peak from impurity peaks and from any interfering peaks arising from excipients. Various types of impurities may be present in drug substances or drug products. These impurities are broadly classified into four main categories: process-related impurities in the drug substance, process-related impurities in the drug product, degradation products of the drug substance, and degradation products of the drug product (Bhutani et al., 2018). Proper identification, separation, and quantification of these impurities are essential to ensure product quality, safety, and regulatory compliance. 1.4.3 Origin of Impurities Sources of Impurity Organic type of impurities can form during the production process or storage of the final API or drug product. These impurities can be nonvolatile type or volatile type and normally include ligands, starting materials, Intermediates compounds of the process, degradation products, reagents, by products from the reactions, and catalysts (Bhutani et al., 2018; Kumar et al., 2020). Inorganic type of impurities can form

during the production process and this type of impurities normally includes reagents used in the process, ligands, heavy metals or other residual materials, inorganic salts. Several impurities can originate during storage of products. To know about these kinds of impurities, normally stability testing was performed to forecast, assess, and to ensure safety 14 of the drug product. In case of drug products sometimes there is possibility of interaction of excipient with the main ingredient to produce an undesirable product which is considered as an impurity. Method should be capable of separating, and accurately, precisely determining all these impurities which can be formed by various modes, then only method can be considered as stability-indicating method

Methodology

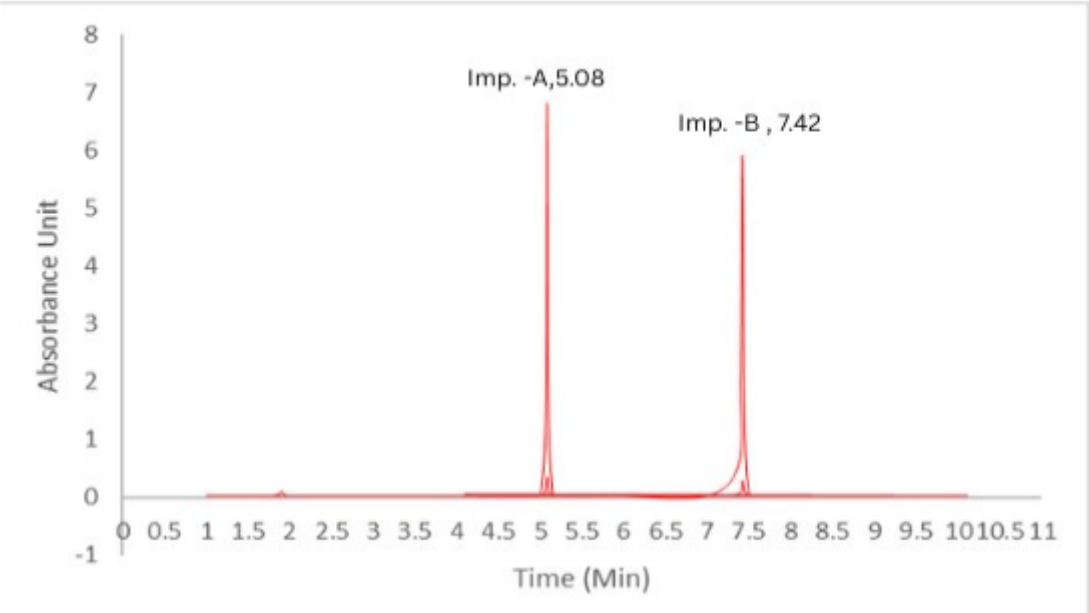
Drug : Favipiarvir ,Molnupiravir

Favipiravir solution was scanned in the UV region between 200–400 nm to determine its absorption characteristics. The UV absorption spectrum showed a prominent peak, indicating significant absorbance of the drug in the UV region. Forced degradation samples such as acid and alkaline treated solutions were also scanned to evaluate spectral changes and possible interference at different wavelengths. It was observed that Favipiravir exhibited maximum absorbance at approximately 320 nm, which was considered as the λmax of the drug. The degradation samples showed comparatively lower absorbance and slight variation in spectral pattern due to structural modification of the drug under stress conditions. However, at 320 nm, interference from degradation products was minimal and the drug response was maximum.is important to assure that once the methods are developed it should reliably and consistently produce precise results with a high degree of accuracy. The method should be specific, and there should not be effect on the results by small changes in the method. This is the basis to validate analytical procedures (ICH, 2005). The validation procedures consist of some parameters along with statistical tools that make the method acceptable. The process of confirming that analytical method is fits for its intended use is called as Method validation (Swartz & Krull, 2012). Results from method validation can be used to judge the quality, reliability, and consistency of analytical results; it is an integral part of any good analytical practice (FDA, 2018). Analytical Method Validation is required in case of new analysis methods or if there are any changes into the analytical procedure, change in the quantitative composition of the drug product and change in synthesis route of the drugs substances (ICH, 2005). 1.6.1 Types of Methods & Validation Parameters Various types of analytical methods are required to be validated in accordance with current regulatory requirements. These methods are generally classified as identification tests, limit tests, and quantitative tests. Identification tests are used to confirm the identity of a substance, while limit tests are performed to control the presence of impurities or other specified components within defined limits. Quantitative tests are designed to determine the exact amount of an analyte present in a sample, such as assay, impurity

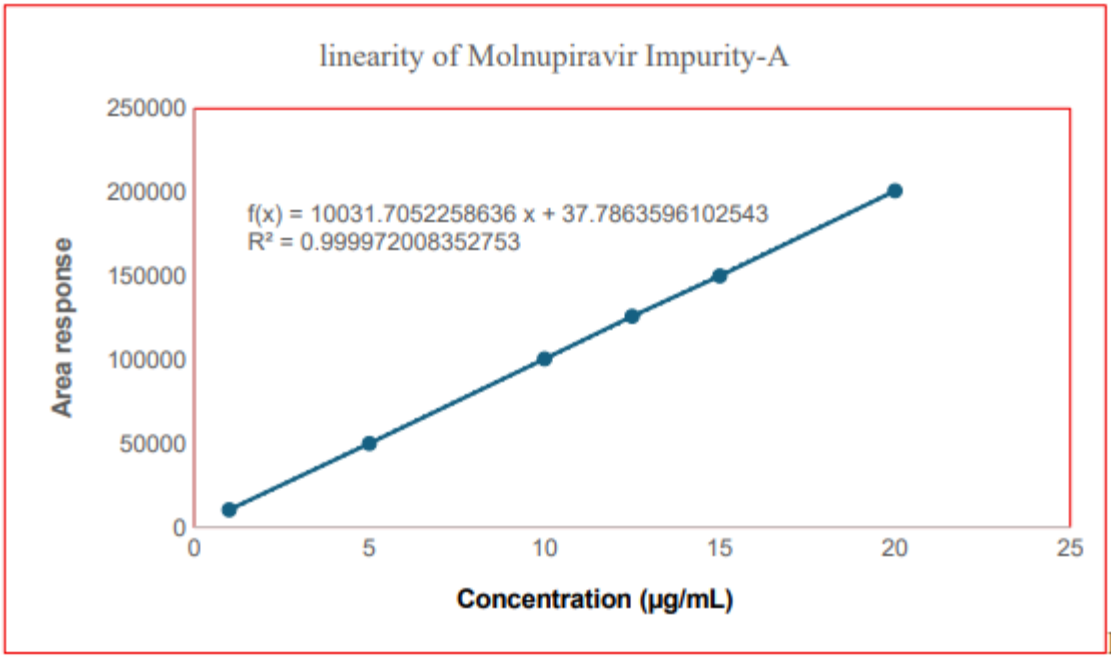
content, or dissolution. For quantitative analytical methods, specific validation characteristics must be evaluated to demonstrate that the method is suitable for its intended purpose. As per current regulatory guidance, the typical validation parameters to be considered include accuracy, 20 specificity, precision, linearity, range, detection limit, quantitation limit, and robustness. Evaluation of these parameters ensures that the analytical method consistently produces reliable, reproducible, and scientifically sound results, thereby supporting product quality and regulatory compliance. 1.7 Need for Advanced Analytical Techniques Continuous advancements in pharmaceutical research, particularly in antiviral therapy, have established a critical demand for advanced analytical techniques. While traditional methods such as titrimetric analysis and basic UV-spectrophotometry remain foundational, they often fail to meet modern requirements for specificity, sensitivity, and simultaneous multi-component analysis in complex formulations. This limitation is especially evident in fixed-dose combinations (FDCs) used in antiretroviral therapy, where traditional methods struggle to accurately quantify individual APIs amidst impurities, excipients, and degradation products. Although conventional High-Performance Liquid Chromatography (HPLC) is widely utilised, it may be limited by longer run times, high solvent consumption, and insufficient resolution for closely related compounds or trace-level impurities. Consequently, modern pharmaceutical analysis increasingly relies on techniques offering higher sensitivity and faster throughput, such as Ultra-High-Performance Liquid Chromatography (UHPLC) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). These advanced tools enable detection at extremely low levels, support stability-indicating capability, and ensure compliance with regulatory requirements for drug safety and efficacy.

Sample Name	Impurity / Compound	Retention Time (min)	Resolution	Purity Angle	Purity Threshold
Specificity Solution	Impurity-A	5.08	6.85	1.42	5.00
Specificity Solution	Impurity-B	7.42	5.63	1.76	5.00

Tab: Impurities retention time

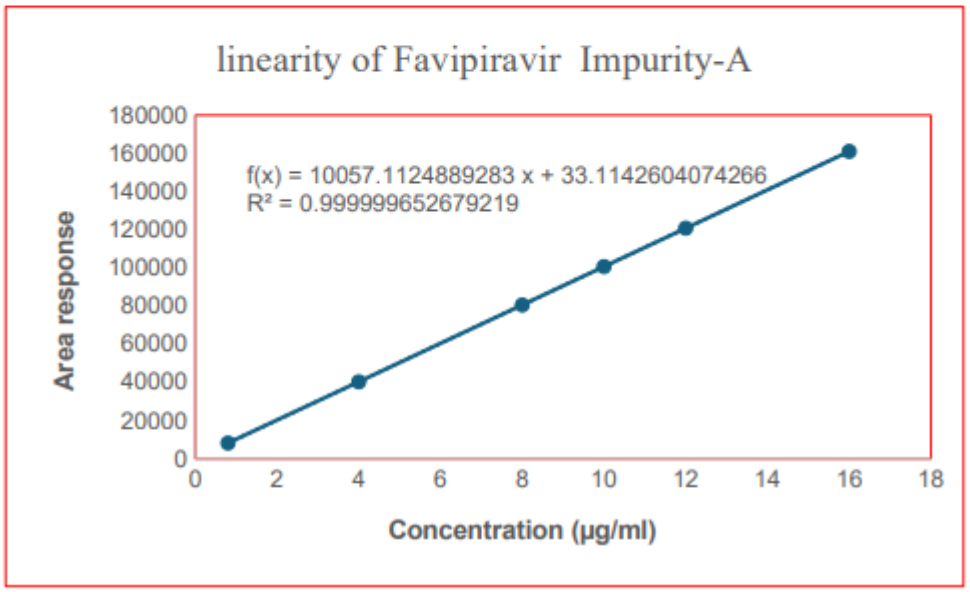


Chromotogram molnupiravir



Sample Name	Impurity / Compound	Retention Time (min)	Resolution	Purity Angle	Purity Threshold
Specificity Solution	Impurity-A	6.18	7.12	1.28	5.00
Specificity Solution	Impurity-B	8.30	5.94	1.61	5.00

Levels (%)	Concentration (µg/mL)	Area Response
LOQ	0.8	8125
50	4.0	40218
100	8.0	80476
125	10.0	100582
150	12.0	120735
200	16.0	160964



Results and discussion

The present work was aimed at the selection of analytical wavelength for the estimation of Molnupiravir by UPLC method. Proper wavelength selection is essential to achieve maximum sensitivity and accurate detection of the drug during chromatographic analysis. Molnupiravir solution was scanned in the UV region between 200–400 nm to determine its absorption characteristics. The UV absorption spectrum showed a prominent peak, indicating significant absorbance of the drug in the UV region. Forced degradation samples such as acid and alkaline treated solutions were also scanned to evaluate spectral changes and possible interference at different wavelengths. It was observed that Molnupiravir exhibited maximum absorbance at 277 nm, which was considered as the λmax of the drug. The degradation samples showed comparatively lower absorbance and slight variation in spectral pattern due to structural modification of the drug under stress conditions. However, at 277 nm, interference from degradation products was minimal and the drug response was maximum. Therefore, 277 nm was selected as the analytical wavelength for further UPLC method development and validation, as it provided better sensitivity, specificity, and reliable estimation of Molnupiravir in the presence of degradation products. The chromatogram of Molnupiravir shows two clearly separated impurity peaks: Imp-A at 5.08 min and Imp-B at 7.42 min. Both peaks are sharp, symmetrical, and well resolved with good baseline separation, indicating effective impurity separation and suitability of the method for related substance analysis. Peak purity of the Molnupiravir peak was evaluated using a Diode Array Detector (DAD). The purity angle was compared with the purity threshold to verify the spectral homogeneity of the peak. No interference from the blank was observed at the retention time of the Molnupiravir peak. No degradant peaks were detected at the retention time of Molnupiravir during degradation and stability studies, indicating that the method is stability-indicating. The purity angle was found to be less than the purity threshold, confirming the absence of spectral co-elution. Furthermore, the resolution between adjacent peaks was greater than 2.0, demonstrating adequate chromatographic separation. Favipiravir solution was scanned in the UV region between 200–400 nm to determine its absorption characteristics. The UV absorption spectrum showed a prominent peak, indicating significant absorbance of the drug in the UV region. Forced degradation samples such as acid and alkaline treated solutions were also scanned to evaluate spectral changes and possible interference at different wavelengths. It was observed that Favipiravir exhibited maximum absorbance at approximately 320 nm, which was considered as the λmax of the drug. The degradation samples showed comparatively lower absorbance and slight variation in spectral pattern due to structural modification of the drug under stress conditions. However, at 320 nm, interference from degradation products was minimal and the drug response was

maximum. Therefore, 320 nm was selected as the analytical wavelength for further UPLC method development and validation, as it provided better sensitivity, specificity, and reliable estimation of Favipiravir in the presence of degradation products.

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