Journal for Research in Applied Sciences and Biotechnology

www.jrasb.com

ISSN: 2583-4053

Volume-2 Issue-2 || April 2023 || PP. 53-60

https://doi.org/10.55544/jrasb.2.2.9

A Comprehensive Review on Analytical Method Development using RP-HPLC and Recent Advances in Pharmaceutical Applications

Deepak Kumar[1], Amrendra Kumar[2], Vinay Kumar[3], Arjesh Raj[4], Raja Ram Mohan Rai[5], Vishal Baliyan[6] and Nitish Kumar[7]

[1]Scholar, Department of Quality Assurance, Roorkee College of Pharmacy, Roorkee, INDIA.
[2]Scholar, Department of Pharmaceutics, Roorkee College of Pharmacy, Roorkee, INDIA.
[3]Faculty, Department of Pharmaceutics, Rajvanshi Ramashish College of Pharmacy, Bihar, INDIA.
[4]Ph.D. Scholar, Department of Pharmacology, Mansarovar Global University, MP, INDIA.
[5]Faculty, Department of Pharmaceutics, Shivam College of Higher Studies Pharmacy, Bihar INDIA.
[6]Faculty, Department of Pharmaceutics, Roorkee College of Pharmacy, Roorkee, INDIA.
[7]Ph.D. Scholar, Department of Pharmaceutics, Lords University Chikani, Alwar, Rajasthan, INDIA.

[1]Corresponding Author: mrdeep15k@gmail.com

www.jrasb.com || Vol. 2 No. 2 (2023): April Issue

Received: 10-03-2023	**Revised:** 31-03-2023	**Accepted:** 10-04-2023

ABSTRACT

The analytical technique of choice for separating, identifying, and quantifying complex mixtures is high-performance liquid chromatography (HPLC). Reverse-phase liquid chromatography (RP-HPLC) is the preferred separation mode for high-performance liquid chromatography (HPLC) due to its adaptability and higher selectivity for hydrophobic compounds. This review article discusses the fundamentals of reversed-phase high-performance liquid chromatography (RP-HPLC). This covers the separation principle, various stationary and mobile phase types, and separation-affecting variables. This article highlights the need of developing and testing such methods in addition to outlining the advantages of using RP-HPLC in industries like pharmaceutical, food, and environmental analysis. As examples of more recent advancements in RP-HPLC, new stationary and mobile phases, RP-HPLC downsizing, and hyphenated methods are also discussed. This review article provides a comprehensive tool for designing, refining, and validating RP-HPLC processes.

Keywords- HPLC, Pharmaceutical Application, Phases, Active Ingredients.

I. INTRODUCTION

High-performance liquid chromatography (HPLC) has established itself as a significant analytical instrument for the separation, identification, and quantification of chemical compounds. The most used HPLC technique is reverse-phase high-performance liquid chromatography (RP-HPLC), which excels in selectivity and sensitivity for a wide range of analytes. Because RP-HPLC can separate compounds based on their hydrophobicity or lipophilicity, it is widely used in pharmaceutical analysis [1].

Without RP-HPLC, it is difficult to separate and quantify APIs and contaminants in pharmaceutical analysis. Robust and accurate RP-HPLC processes must be created in order to assess the drug's quality, safety, and effectiveness. In order to establish analytical methodologies using RP-HPLC, it is essential to choose the stationary and mobile phases, optimize the separation conditions, and choose the chromatographic parameters [2].

In this review article, the foundations of reversed-phase high-performance liquid chromatography (RP-HPLC), including the separation principle, alternatives for stationary and mobile phases, and

ISSN: 2583-4053

Volume-2 Issue-2 || April 2023 || PP. 53-60

https://doi.org/10.55544/jrasb.2.2.9

variables affecting RP-HPLC separation, are discussed [3]. We will also discuss the many steps involved in the development and validation of RP-HPLC methods and highlight the many applications of RP-HPLC, including pharmaceutical analysis, food analysis, and environmental analysis. Along with other recent advancements in RP-HPLC, new stationary and mobile phases, RP-HPLC downsizing, and hyphenated techniques like LC-MS [4] will also be addressed.

The pharmaceutical sector has found RP-HPLC to be crucial since it can separate and quantify impurities and active pharmaceutical ingredients (APIs). The effectiveness and selectivity of RP-HPLC have improved recently as a result of developments in both stationary and mobile phases. Miniaturization advances have made it possible to analyze smaller sample quantities with RP-HPLC at a lower cost and in less time. The creation of hybrid techniques like LC-MS has led to increased sensitivity and selectivity in RP-HPLC analysis [5].

In conclusion, the pharmaceutical business relies heavily on RP-HPLC as an analytical tool, hence it's crucial that reliable and precise RP-HPLC techniques be created to assess medications. The effectiveness, sensitivity, and selectivity of RP-HPLC have recently improved, making it a more useful tool for the analysis of chemical compounds in a variety of fields [6].

II. FUNDAMENTALS OF RP-HPLC

A number of chromatographic parameters are also used to gauge the effectiveness of RP-HPLC separations. The retention time is the length of time it takes for a molecule to elute from the column after injection, whereas the selectivity factor is the ratio of the distance between two peaks. Resolution measures the space between two successive peaks, while capacity factor measures the compound's relative affinity for the stationary phase [7].

The first stage in developing a successful separation using the RP-HPLC technology is selecting the stationary and mobile phases. It is crucial to pick a stationary phase that works well with the mobile phase and can successfully trap the target analyte. It's critical to choose a mobile phase that elutes the target analyte from the column and is compatible with the stationary phase [8].

To get the best separation conditions, it is possible to adjust a number of parameters, including column temperature, mobile phase pH, flow rate, and gradient elution. The optimization process aims to achieve maximum resolution, minimal analytical time, and optimal solvent consumption [9]. To guarantee that RP-HPLC methods are reliable, reproducible, and precise, validation is crucial. Some of the factors that are investigated during validation include accuracy, precision, linearity, resilience, and limitations of detection and quantification [10].

Just a few of the several industries that can profit from RP-HPLC include the examination of food, pharmaceuticals, and the environment. Impurities and degradation products are identified and separated utilizing RP-HPLC analysis of drug molecules and drug products. The chemical composition and general quality of a product are frequently examined using RP-HPLC in the food business. RP-HPLC can be used to identify and measure pollutants in environmental samples [11].

Recent advancements in RP-HPLC include its downsizing, the introduction of hyphenated methodologies like LC-MS, and the creation of new stationary and mobile phases. More precise separation and analysis of complex compounds may be possible with newly constructed stationary and mobile phases with increased selectivity and efficiency [12]. Miniaturized RP-HPLC can analyze smaller sample volumes, reducing run durations and solvent consumption. By combining the advantages of RP-HPLC with mass spectrometry, hybrid techniques provide more accurate and sensitive analyte detection [13].

In conclusion, RP-HPLC is a vital analytical chemistry technique, particularly for the investigation of medicines. The creation of accurate and dependable RP-HPLC techniques is necessary for determining the quality, safety, and efficacy of drug products [14]. The stationary and mobile phases must be carefully considered, the separation parameters must be fine-tuned, and RP-HPLC methodologies must be validated in order to achieve the perfect separation. One way that RP-HPLC is changing to become more adaptable and potent in the analytical environment is through the addition of new stationary and mobile phases, downsizing, and hyphenated techniques [15].

III. METHOD DEVELOPMENT IN RP-HPLC

The method development process involves selecting appropriate stationary and mobile phases, optimizing separation conditions, and determining chromatographic parameters. The selection of stationary and mobile phases is based on the physicochemical properties of the sample components, such as polarity, solubility, and acid-base properties. The optimization of separation conditions involves adjusting the pH, solvent strength, and temperature to achieve the desired separation [16].

In the method development process, the selection of stationary and mobile phases is critical in achieving the desired separation. The stationary phase is typically a hydrophobic material, such as C18, C8, or phenyl, which interacts with the analytes based on their hydrophobicity [17]. The mobile phase, on the other hand, is typically a mixture of an aqueous phase and an

ISSN: 2583-4053

Volume-2 Issue-2 || April 2023 || PP. 53-60

https://doi.org/10.55544/jrasb.2.2.9

organic solvent, such as acetonitrile or methanol. The choice of the mobile phase composition depends on the sample's properties and the desired separation [18].

The optimization of separation conditions involves adjusting the pH, solvent strength, and temperature to achieve the desired separation. The pH of the mobile phase can affect the ionization of the analytes and the degree of retention on the column. Solvent strength is controlled by adjusting the ratio of the organic solvent to the aqueous phase, and temperature can affect the selectivity and resolution of the separation [19].

Once the separation conditions are optimized, the determination of chromatographic parameters is crucial in validating the method's reliability and accuracy. Retention time is the most common chromatographic parameter and is used to identify the analytes in the sample. Selectivity is a measure of the separation between two analytes and is calculated by measuring the relative retention times of the two analytes. Resolution is a measure of the separation between two adjacent peaks, and it is calculated by measuring the peak widths and distances between the two peaks [20].

In summary, the method development process in RP-HPLC involves selecting appropriate stationary and mobile phases, optimizing separation conditions, and determining chromatographic parameters to achieve a reliable and accurate separation of the analytes. The optimization of separation conditions and the determination of chromatographic parameters are critical steps in ensuring the method's reliability and accuracy in pharmaceutical analysis [21].

IV. VALIDATION OF RP-HPLC METHODS

Method validation is essential to ensure the reliability and accuracy of RP-HPLC methods. The validation process involves evaluating the parameters of accuracy, precision, linearity, and robustness. Accuracy is the closeness of the measured value to the true value, and precision is the degree of reproducibility of the results [22]. Linearity is the ability of the method to produce a linear response over a range of concentrations, and robustness is the ability of the method to produce consistent results despite small changes in the method parameters. Validation of RP-HPLC methods is a critical step in the development of reliable and accurate analytical methods. The validation process evaluates the performance of the method and verifies that it is suitable for its intended use. The various parameters evaluated during method validation are accuracy, precision, linearity, and robustness [23].

Accuracy

In RP-HPLC, accuracy and precision are critical parameters in method validation as they reflect the reliability and reproducibility of the analytical method. Accuracy is often assessed by spiking the sample with a known amount of the analyte of interest and comparing the measured value with the expected value [24]. This approach is particularly useful when the true value of the analyte is unknown or when the sample matrix is complex, which may affect the recovery of the analyte. The accuracy can be expressed as the percentage of recovery or the absolute difference between the measured and expected values [25].

In contrast, precision is the degree of reproducibility of the results obtained from multiple measurements of the same sample. It is often evaluated by calculating the relative standard deviation (RSD) of the results, which is expressed as the ratio of the standard deviation to the mean value of the measurements. A low RSD indicates a high degree of precision, which is desirable for analytical methods [26].

Both accuracy and precision are influenced by several factors, including the quality of the analytical instruments, the performance of the chromatographic system, the stability of the analyte, and the skill level of the operator [27]. To ensure accurate and precise results, it is crucial to validate the method before its application in the analysis of real samples. Method validation involves assessing several parameters, including linearity, limit of detection, limit of quantification, specificity, robustness, and ruggedness, among others. By validating the method, the analyst can ensure that the method is fit for the intended purpose and that the results obtained are reliable and reproducible [28].

Linearity

Linearity is an essential aspect of method validation in RP-HPLC analysis. It is necessary to determine if the response of the detector is proportional to the concentration of the analyte. This parameter is crucial because it ensures that the method can produce accurate and reliable results over a range of concentrations [29]. To assess linearity, a series of standard solutions with known concentrations of the analyte should be prepared. The standard solutions should cover a range of concentrations that is relevant to the analysis. The peak area or peak height should be measured for each solution, and the data should be plotted to generate a calibration curve [30].

A linear correlation between the peak area or height and the concentration should be observed, with a correlation coefficient of at least 0.99. If the correlation coefficient is lower than 0.99, the method may need to be optimized or further validated to ensure that it produces accurate and reliable results [31]. In addition to determining linearity, the calibration curve can also be used to calculate the limit of detection (LOD) and limit of quantitation (LOQ) for the method. The LOD and LOQ represent the lowest concentration of the analyte that can be detected and quantified, respectively. Overall, linearity is a critical parameter in method validation, and it is essential to ensure that the method can produce accurate and reliable results over a range of concentrations [32].

ISSN: 2583-4053

Volume-2 Issue-2 || April 2023 || PP. 53-60

https://doi.org/10.55544/jrasb.2.2.9

Robustness

Robustness is a critical parameter in the validation of RP-HPLC methods, as it indicates the method's ability to produce consistent results despite minor variations in the experimental conditions. To evaluate the robustness of an RP-HPLC method, various parameters such as pH, temperature, and flow rate are intentionally varied, and the effects on the results are observed [33]. If the changes in the parameters do not significantly affect the results, then the method is considered to be robust. A robust method is highly desirable as it ensures that the results obtained are reliable and reproducible [34].

In addition to robustness, other parameters such as accuracy, precision, and linearity are also essential to validate the RP-HPLC method's performance. Accuracy refers to the closeness of the measured values to the true values, while precision measures the degree of reproducibility of the results. Linearity, on the other hand, evaluates the method's ability to produce a linear relationship between the analyte concentration and the response [35].

Overall, a comprehensive assessment of the RP-HPLC method's performance is necessary to verify its suitability for the intended use. Validation of the method ensures the reliability and accuracy of the results, and it also helps to identify any potential issues with the method, allowing for adjustments to be made to improve its performance [36].

V. APPLICATIONS OF RP-HPLC IN PHARMACEUTICAL ANALYSIS

RP-HPLC is a widely used technique in the pharmaceutical industry for drug analysis due to its high sensitivity, selectivity, and reproducibility. The application of RP-HPLC in pharmaceutical analysis involves the separation and quantification of active pharmaceutical ingredients (APIs), impurities, degradation products, and other related substances [37].

One of the primary applications of RP-HPLC in pharmaceutical analysis is the determination of drug purity. This involves the separation of the API from other components in the sample and quantifying the amount of the API present. The analysis of drug impurities is also an essential application of RP-HPLC. Impurities can arise from various sources, such as starting materials, intermediates, and degradation products. RP-HPLC is capable of separating these impurities from the API and determining their concentration in the drug substance [38].

Another significant application of RP-HPLC in pharmaceutical analysis is the analysis of drug formulations. This involves the analysis of the active ingredients and excipients in a drug product. RP-HPLC is used to separate the active ingredient from the excipients, and the amount of the API present in the formulation is quantified [39]. RP-HPLC is also used in the analysis of drug degradation products. The degradation of drugs can occur due to various factors such as temperature, light, and moisture. RP-HPLC can be used to separate and quantify the degradation products formed during storage or during the manufacturing process [40].

RP-HPLC is also used in the analysis of drug-drug interactions. This involves the separation and quantification of the drug and its metabolites in biological matrices, such as plasma or urine. The analysis of drug-drug interactions is essential to evaluate the safety and efficacy of drugs and to determine the pharmacokinetic parameters of drugs [41]. In addition to the above applications, RP-HPLC is also used in the analysis of drug stability, bioavailability, and pharmacokinetics. The stability of drugs is determined by subjecting the drug substance to various stress conditions and analyzing the resulting degradation products. RP-HPLC is used to separate and quantify the degradation products formed during the stability studies. The bioavailability and pharmacokinetics of drugs are also determined by RP-HPLC analysis of biological matrices such as plasma or urine [42].

In conclusion, RP-HPLC is an essential technique in pharmaceutical analysis due to its high sensitivity, selectivity, and reproducibility. The applications of RP-HPLC in pharmaceutical analysis include drug purity determination, impurity analysis, analysis of drug formulations, analysis of drug degradation products, analysis of drug-drug interactions, drug stability studies, and determination of bioavailability and pharmacokinetics of drugs [43].

VI. RECENT ADVANCEMENTS IN RP-HPLC

Prajapati, P. B., et al., (2021), in recent years, the development of stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) methods has been a topic of interest in the pharmaceutical industry. Bosutinib, a tyrosine kinase inhibitor used for the treatment of chronic myelogenous leukemia, is a compound for which no stability-indicating RP-HPLC method has been reported in the literature. To address this, a stability-indicating RP-HPLC method for the estimation of bosutinib has been developed using a risk- and design of experiments (DoE)-based enhanced analytical quality by design (AQbD) approach. The method involves chromatographic separation on a C18 column using acetonitrile–1.0% triethylamine (v/v) in water (pH 7.0). The developed method was validated according to International Conference on Harmonization (ICH) Q2 (R1) guidelines and applied to assay pharmaceutical dosage forms and in an oxidative degradation kinetic study of bosutinib at different pH conditions. The method provides a valuable analytical tool for quality control and stability studies of pharmaceutical dosage forms of bosutinib in the pharmaceutical industry [44].

ISSN: 2583-4053

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https://doi.org/10.55544/jrasb.2.2.9

Ibrahim, F. A., et al., (2019), the use of eco-friendly analytical methods has gained momentum in different fields, including pharmaceutical analysis. However, most chromatographic techniques still employ significant amounts of toxic and non-degradable organic solvents. This review highlights a green HPLC assay method for the simultaneous estimation of two moxifloxacin combinations: moxifloxacin/dexamethasone and moxifloxacin/prednisolone. The method involves the use of a reversed-phase Thermo Scientific MOS-1 Hypersil C8 column (250 mm × 4.6 mm i.d., 5-µm particle size) and an eco-friendly isocratic eluent composed of ethanol: water containing 0.05% triethanolamine (90:10, v/v, pH 4.5). The assay was completed in less than 6 min for each combination at a flow rate of 1.0 mL/min. A UV detector was used at 240 nm for the first 4.0 min and later at 280 nm using time programming technique. The method enabled the simultaneous analysis of each of moxifloxacin combinations in their laboratory-prepared binary mixtures in various pharmaceutical ratios. Additionally, the method was used to analyze eye drops containing either moxifloxacin/dexamethasone or moxifloxacin/prednisolone with success. The proposed method was also evaluated for its greenness using three different tools and was found to be an eco-friendly alternative to conventionally developed HPLC methods regarding the usage of safe solvents and chemicals, minimal waste production, and short analysis time. Therefore, the method could be useful for routine quality control analysis of the investigated binary mixtures with the least harmful effect on the environment or human beings [45].

Attimarad, M., et al., (2020), the objective of this literature review was to evaluate the recent advancements in RP-HPLC for the development of a rapid, economical, and robust liquid chromatographic procedure for the quality control of a pharmaceutical preparation containing amlodipine and celecoxib using an experimental design. The proposed method involved isocratic separation using a Zorbax C18 (150 mm) RP-HPLC column and a mobile phase consisting of sodium phosphate buffer (pH 5.6): acetonitrile: methanol in a ratio of 30:55:15 (v/v). The analysis was performed at 25°C with a flow rate of 1.2 mL/minute and detection at 239 nm using etoricoxib as an internal standard. The calibration curve showed good linearity, and the precision and accuracy were within an acceptable range. The suggested procedure was established by the results of recovery studies using a standard addition method. The robustness test was performed using fractional factorial design with three major contributing factors, demonstrating that the pH of the mobile phase and flow rate had a substantial effect on the peak area ratio of amlodipine, while the percent of acetonitrile and the flow rate together exhibited an effect on the peak area ratio of celecoxib. The validated, robust analytical method has been utilized to quantify amlodipine and celecoxib from formulations with large concentration differences, showing no significant difference in terms of accuracy and precision when compared with reported methods [46].

Prajapati, P., et al., (2022), The recent development of multipurpose reverse-phase high-performance liquid chromatography (RP-HPLC) method has gained significant interest in the pharmaceutical industry for its economical and eco-friendly alternative to numerous published chromatography methods for synchronous estimation of FDC of telmisartan to save time, cost and solvent for analysis. In this regard, the analytical quality risk management was initiated with identification of potential method parameters using cause–effect diagram followed by assessment of their risk by risk priority number ranking and filtering method. To control the risk of critical method parameters, their optimization using design of experiments-based full-factorial design was employed. The method operable design ranges were identified for high-risk method parameters, and control strategy was set for mitigation of their risk throughout the life cycle of the developed RP-HPLC method. The RP-HPLC method was validated as per the ICH Q2 (R1) guideline and applied for simultaneous estimation of seven FDC products of telmisartan, complying with labeled claim. The study highlights the potential of the developed RP-HPLC method as a multipurpose RP-HPLC method for quality control of FDC products of telmisartan in the pharmaceutical industry to save solvent, cost and time of analysis [47].

Darwish, H. W., et al., (2021), the use of RP-HPLC in pharmaceutical analysis has continued to grow due to its selectivity, sensitivity, and stability-indicating properties. One recent advancement in RP-HPLC is the establishment of a reliable and sensitive stability-indicating method for the quantification of bromazepam and one of its degradants, 2-(2-amino-5-bromobenzoyl) pyridine (ABP). The study utilized a C18 column and methanol-water (70:30, v/v) as the mobile phase, with detection achieved by a photodiode array detector set at 230 nm. The method was validated following ICH guidelines, with the calibration curves of BMZ and ABP created in the range of 1-16 µg mL-1 and a mean recovery percentage of 100.02 ± 1.245 and 99.74 ± 1.124, respectively. BMZ stability was inspected under various ICH forced degradation conditions, revealing its susceptibility to degradation in acidic and alkaline conditions. The method was shown to be suitable for quantifying the impurity (ABP) in a BMZ pure sample and for determining BMZ in pharmaceutical dosage forms, with no significant differences compared to a reference HPLC method in regard to both accuracy and precision. This study highlights the potential of RP-HPLC in developing stability-indicating methods for the quantification of drug substances and their impurities [48].

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https://doi.org/10.55544/jrasb.2.2.9

Jin, P., et al., (2021), in recent years, the analysis of microcystin-LR (MC-LR) has gained significant attention in the field of environmental monitoring due to its potential threat to human health. The development and comparison of analytical methods for detecting MC-LR in a wide concentration range is crucial for accurate and precise quantification. This literature review focuses on the recent advancements in RP-HPLC and UPLC-MS for the determination of MC-LR. The study proposes the combined use of UPLC-MS and HPLC-VWD approaches for the detection of MC-LR. UPLC-MS was utilized to determine MC-LR at trace concentrations due to its high sensitivity, whereas HPLC-VWD was used for the determination of high concentrations. The linear ranges of UPLC-MS and HPLC-VWD methods were 0.08-10 μg L−1 and 1-5000 μg L−1, respectively. The detection and quantification limits of UPLC-MS were found to be 0.03-0.05 μg L−1 and 0.08 μg L−1, respectively, and those of HPLC-VWD were 0.6 and 1.0 μg L−1. The sensitivity, precision, and accuracy of the two methods were compared in detail, and the results indicated that both methods were reliable and effective for the detection of MC-LR. The study also evaluated the potential adsorption properties of MC-LR on filter membranes and found no significant effects. The proposed methods were applied to water samples from Erhai Lake, China, and the results demonstrated the applicability and effectiveness of the combined approach for the determination of MC-LR in real environmental samples [49].

VII. CONCLUSION

In conclusion, RP-HPLC is an essential analytical technique in pharmaceutical analysis. Method development using RP-HPLC involves selecting appropriate stationary and mobile phases, optimizing separation conditions, and determining chromatographic parameters. Method validation is crucial to ensure the reliability and accuracy of RP-HPLC methods. RP-HPLC has several applications in pharmaceutical analysis and other fields, and recent advancements in RP-HPLC offer higher efficiency, faster separations, and more accurate and sensitive detection. Future perspectives on RP-HPLC method development include the use of more sustainable and eco-friendly stationary and mobile phases and the development of automated and high-throughput methods.

REFERENCES

[1] A Suganthi, CHS Lakshmi, S Vinod, et al. "Development of validated RP-HPLC method for bosentan in formulation and its application to in vitro interaction study with aceclofenac." Journal of Pharmaceutical Analysis, vol. 4, no. 5, 2014, pp. 363-371. doi: 10.1016/j.jpha.2014.03.003.

[2] H Mustafa. "Method Development and Validation for the Simultaneous Estimation of Ambrisentan in Tablet Dosage form by HPLC." Tamil Nadu Dr. M.G.R. Medical University, 2016.

[3] S Muralidharan, JR Kumar. "Simple estimation of bosentan in tablet formulation by RP-HPLC." Open Journal of Applied Sciences, vol. 2, no. 4, 2012, pp. 242-246. doi: 10.4236/ojapps.2012.24037.

[4] K Lingamaneni, MM Annapurna. "Development and validation a new stability indicating RP-UFLC method for the estimation of Bosentan." Research Journal of Pharmacy and Technology, vol. 14, no. 3, 2021, pp. 1463-1468. doi: 10.5958/0974-360X.2021.00262.4.

[5] S Orlandini, B Pasquini, C Caprini, M Del Bubba, et al. "Enantioseparation and impurity determination of ambrisentan using cyclodextrin-modified micellar electrokinetic chromatography: Visualizing the design space within quality by design approach." Journal of Chromatography A, vol. 1438, 2016, pp. 75-84. doi: 10.1016/j.chroma.2016.01.039.

[6] VSK Yelamanchi. "Modified and residual determination method development and validations for active pharmaceuticals ingredient by RP HPLC." KL University, 2017.

[7] KS Nataraj, GN Raju, AS Rao, SVS Kumar, S Divya. "European Journal of Pharmaceutical and Medical Research." European Journal of Pharmaceutical and Medical Research, vol. 4, no. 5, 2017, pp. 28-36.

[8] Sultana, A., Singh, M., Kumar, A., Kumar, R., Saha, P., Kumar, R. S., & Kumar, D. (2022). To Identify Drug-Drug Interaction in Cardiac Patients in Tertiary Care Hospitals. *Journal for Research in Applied Sciences and Biotechnology*, *1*(3), 146-152.

[9] RC Prabhu, A Maruthapillai, GC Senadi. "Wise AQbD and an Orthogonality Approach to Develop a Short-Runtime Method for the Simultaneous Quantification of Bosentan and Impurities using UPLC Equipped." Analytical Chemistry, vol. 93, no. 6, 2021, pp. 3041-3049. doi: 10.1021/acs.analchem.0c05159.

[10] S Sharmin, MH Sohrab, F Moni, F Afroz, et al. "Simple RP-HPLC method for Aceclofenac quantitative analysis in pharmaceutical tablets." Pharmacia, vol. 67, no. 2, 2020, pp. 79-85. doi: 10.3897/pharmacia.67.e51691.

[11] SA Jadhav, SB Landge, SL Jadhav, et al. "Stability-indicating gradient RP-LC method for the determination of process and degradation impurities in bosentan monohydrate: An endothelin receptor antagonist." Chromatography Research International, vol. 2011.

[12] Kirthi, A., Shanmugam, R., Prathyusha, M. S., & Kumar, S. S. (2014). A review on bioanalytical method development and validation by RP-HPLC. Journal of global trends in pharmaceutical sciences, 5(4), 2028-2037.

[13] Pyrzynska, K., & Sentkowska, A. (2015). Recent developments in the HPLC separation of phenolic food

ISSN: 2583-4053

Volume-2 Issue-2 || April 2023 || PP. 53-60

https://doi.org/10.55544/jrasb.2.2.9

compounds. Critical Reviews in Analytical Chemistry, 45(1), 41-51.

[14] Liang, C., & Lian, H. (2015). Recent advances in lipophilicity measurement by reversed-phase high-performance liquid chromatography. TrAC Trends in Analytical Chemistry, 71, 63-73.

[15] Keshri, S., Kumar, R., Kumar, D., Singhal, T., Giri, S., Sharma, I., & Vatsha, P. (2022). Insights Of Artificial Intelligence In Brain Disorder With Evidence Of Opportunity And Future Challenges. *Journal of Pharmaceutical Negative Results*, 10853-10867.

[16] Krokhin, O. V. (2006). Sequence-specific retention calculator. Algorithm for peptide retention prediction in ion-pair RP-HPLC: application to 300-and 100-Å pore size C18 sorbents. Analytical chemistry, 78(20), 7785-7795.

[17] Matsuda, Y., & Mendelsohn, B. A. (2021). Recent Advances in Drug–Antibody Ratio Determination of Antibody–Drug Conjugates. Chemical and Pharmaceutical Bulletin, 69(8), 680-694.

[18] Edla, S., & Sundhar, B. S. (2014). Analytical method development and validation for the simultaneous estimation of Metformin and Glibenclamide in bulk and tablet dosage form using RP-HPLC. Rasayan journal of chemistry, 7(4), 389-394.

[19] Welch, C. J., Brkovic, T., Schafer, W., & Gong, X. (2009). Performance to burn? Re-evaluating the choice of acetonitrile as the platform solvent for analytical HPLC. Green Chemistry, 11(11), 1704-1708.

[20] Zaman, B., Siddique, F., & Hassan, W. (2016). RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application to in vitro dissolution studies. Chromatographia, 79(23-24), 1595-1602.

[21] Kavitapu, D. R., Maruthapillai, A., Mahapatra, S., Jetti, R. K., & Sirisha, K. (2021). New stability indicating RP-HPLC method for the determination of Abiraterone acetate, its related substances and degradation products in bulk and dosage form. Materials Today Proceedings, 46, 6223-6231.

[22] Purabisaha, R. K., Rawat, S. S. N., & Prakash, A. (2021). A Review On Novel Drug Delivery System.

[23] Jahan, M. S., Islam, M. J., Begum, R., Kayesh, R., & Roy, B. (2014). Method development, validation, and forced degradation for simultaneous quantification of paracetamol and ibuprofen in pharmaceutical dosage form by RP-HPLC. Analytical chemistry insights, 9, 17-27.

[24] Ali, SNS, Mobina, L, Mehfuza, M. (2021). Analytical method development and validation and forced degradation stability-indicating studies of Favipiravir by RP-HPLC and UV in bulk and pharmaceutical. Journal of Pharmaceutical Research International, 33(5), 1-13.

[25] Nirupa, G., Tripathi, UM. (2013). RP-HPLC analytical method development and validation for simultaneous estimation of three drugs: Glimepiride, pioglitazone, and metformin and its …. Journal of Chemistry, 2013, 1-7.

[26] Pedroso, TM, Medeiros, ACD, Salgado, HRN. (2016). RP-HPLC× HILIC chromatography for quantifying ertapenem sodium with a look at green chemistry. Talanta, 161, 882-888.

[27] Yabré, M., Ferey, L., Somé, IT., Gaudin, K. (2018). Greening reversed-phase liquid chromatography methods using alternative solvents for pharmaceutical analysis. Molecules, 23(4), 780.

[28] Umama, Y., Venkatajah, G., Shourabh, R., Kumar, R., Verma, A., Kumar, A., & Gayoor, M. K. (2019). Topic-The scenario of pharmaceuticals and development of microwave as; sisted extraction technique. *World J Pharm Pharm Sci*, *8*(7), 1260-1271.

[29] Tome, T., Žigart, N., Časar, Z., Jereb, M., Prošek, M. (2019). Development and optimization of liquid chromatography analytical methods by using AQbD principles: Overview and recent advances. Organic Process Research & Development, 23(6), 1196-1206.

[30] Steinmann, D., Ganzera, M. (2011). Recent advances on HPLC/MS in medicinal plant analysis. Journal of Pharmaceutical and Biomedical Analysis, 55(4), 744-757.

[31] Navas, N., Herrera, A., Martínez-Ortega, A., Zafra-Gómez, A., González-Ruiz, V. (2013). Quantification of an intact monoclonal antibody, rituximab, by (RP) HPLC/DAD in compliance with ICH guidelines. Analytical and Bioanalytical Chemistry, 405(19), 6333-6341.

[32] Liu, DQ., Sun, M., Kord, AS. (2010). Recent advances in trace analysis of pharmaceutical genotoxic impurities. Journal of Pharmaceutical and Biomedical Analysis, 53(4), 519-534.

[33] Cielecka-Piontek, J., Michalska, K., Żelaszczyk, D. (2011). Recent advances in stability studies of carbapenems. Current Pharmaceutical Analysis, 7(3), 186-194.

[34] Attimarad, M., Elgorashe, REE., Subramaniam, R., Zayed, M., Tawakkal, ISMA., Ismail, N., Aboul-Enein, HY. (2020). Development and validation of rapid RP-HPLC and green second-derivative UV spectroscopic methods for simultaneous quantification of metformin and remogliflozin …. Separations, 7(3), 40.

[35] Kumar, R., Saha, P., Sarkar, S., Rawat, N., & Prakash, A. (2021). A Review On Novel Drug Delivery System. *IJRAR-International Journal of Research and Analytical Reviews (IJRAR)*, *8*(1), 183-199.

[36] Swarupa, M.P.G. "A new analytical stability-indicating methods for the development and validation of some pharmaceutical drugs using RP-HPLC." (2019): ir.kluniversity.in.

[37] Arun, B.R., Seema, G., and Bagul, R. "Analytical method development and validation of antihistamine drugs bilastine and montelukast sodium by RP-HPLC and UV spectrophotometric method." International Journal of Pharmaceutical Research and Allied Sciences 6.3 (2017): 44-52.

ISSN: 2583-4053

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https://doi.org/10.55544/jrasb.2.2.9

[38] Hicks, M.B., Oriana, S., and Liu, Y. "Assessment of Analytical Testing: The Impact of Metrics for the Sustainable Measurement of Pharmaceuticals." Current Opinion in Green and Sustainable Chemistry 32 (2022): 100488.

[39] Wimalasinghe, R.M., Zhao, D., Wang, L., et al. "Development and validation of a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for identification, assay, and quantification of sulfobutylether-β-cyclodextrin sodium (SBECD) in drug products." Journal of AOAC International 104.3 (2021): 887-894.

[40] Deore, K.R. "Method development and validation for Apremilast by RP-HPLC." International Journal of Advanced Engineering and Management Research 2.2 (2017): 68-72.

[41] Annadi, A.M., El Zahar, N.M., Abdel-Sattar, NEDA., et al. "Development and validation of molnupiravir assessment in bulk powder and pharmaceutical formulation by the RP-HPLC-UV method." RSC Advances 12 (2022): 1725-1731.

[42] Mangla, B., Beg, S., Alam, O., Ahsan, W., Haque, A., Patel, K. S., ... & Kohli, K. (2020). Systematic development and validation of RP-HPLC method for simultaneous estimation of tamoxifen and sulphoraphane with specific application for nanolipidic formulations. *Arabian Journal of Chemistry*, *13*(11), 7909-7920.

[43] Legrae, L. H., Deida, M. F., Abdellahi, B. M., Elkory, M. B., Ndiaye, I., & Bouajila, J. (2020). An easy efficient method of veterinary drug residue analysis in raw milk by RP-HPLC-UV with application to raw milk. *Current Pharmaceutical Analysis*, *16*(7), 942-949.

[44] Jain, A., Beg, S., Saini, S., Sharma, T., Katare, O. P., & Singh, B. (2019). Application of chemometric approach for QbD-enabled development and validation of an RP-HPLC method for estimation of methotrexate. *Journal of Liquid Chromatography & Related Technologies*, *42*(15-16), 502-512.

[45] Maljurić, N., Otašević, B., Golubović, J., Krmar, J., Zečević, M., & Protić, A. (2020). A new strategy for development of eco-friendly RP-HPLC method using Corona Charged Aerosol Detector and its application for simultaneous analysis of risperidone and its related impurities. *Microchemical Journal*, *153*, 104394.

[46] Sankar, R., Snehalatha, K. S., Firdose, S. T., & Babu, P. S. (2019). Applications in HPLC in pharmaceutical analysis. *International Journal of Pharmaceutical Sciences Review and Research*, *59*, 117-124.

[47] Peram, M. R., Patil, S. R., Kumbar, V. M., Kugaji, M. S., Bhat, K. G., Diwan, P. V., & Jalalpure, S. (2021). An RP-HPLC method for quantitative analysis of linagliptin entrapped in nanotransfersomes and its application to skin permeation studies. *Current Pharmaceutical Analysis*, *17*(2), 231-240.

[48] Mahrouse, M. A., & Lamie, N. T. (2019). Experimental design methodology for optimization and robustness determination in ion pair RP-HPLC method development: Application for the simultaneous determination of metformin hydrochloride, alogliptin benzoate and repaglinide in tablets. *Microchemical Journal*, *147*, 691-706.

[49] Kalyankar, G. (2021). Implementation of DoE and risk-based enhanced analytical quality by design approach to stability-indicating RP-HPLC method for stability study of bosutinib. Journal of AOAC International, 104(6), 1742-1753.