A Comprehensive Review on: RP HPLC Method Development and Validation of Fexofenedine Hydrochloride by Central Composite Design (Qbd)

Chaitali U. Raut[1], Mr. Amol N. Lonarkar[2]
[1]*Student, Shreeyash Institute of Pharmaceutical Education and Research*
[2] *Principal, Shreeyash Institute of Pharmacy*

***Abstract*—Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is extensively utilized in pharmaceutical analysis because of its high sensitivity, accuracy, and reproducibility. The present review article provides a comprehensive overview of the analytical methods reported for the estimation of Fexofenadine Hydrochloride in pharmaceutical formulations using RP-HPLC techniques. Particular attention has been focused on the implementation of Analytical Quality by Design (AQbD) concepts and Central Composite Design (CCD) for systematic method optimization. Various chromatographic conditions, including selection of mobile phase, column type, flow rate, pH adjustment, and detection wavelength, have been critically examined from published literature. In addition, important validation parameters such as accuracy, precision, linearity, robustness, specificity, limit of detection, and limit of quantification have been reviewed in accordance with International Council for Harmonisation (ICH) guidelines. Literature findings reveal that the application of CCD in RP-HPLC method development enhances analytical performance by improving robustness and minimizing experimental variations. Overall, the reviewed studies demonstrate that CCD-based RP-HPLC methods are reliable, economical, and suitable for routine quality control analysis of Fexofenadine Hydrochloride in pharmaceutical dosage forms. The integration of experimental design approaches into analytical method development offers an efficient and scientific pathway for achieving robust pharmaceutical analytical methods.**

***Index Terms*—RP-HPLC, Fexofenadine Hydrochloride, Analytical Quality by Design (AQbD), Central Composite Design (CCD), Method Development, Validation, Pharmaceutical Analysis.**

I. INTRODUCTION

Pharmaceutical analysis plays a vital role in ensuring the quality, safety, and efficacy of drug products through the development of accurate and reliable analytical methods. Among the various analytical techniques available, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) has become one of the most widely used methods in pharmaceutical industries and research laboratories because of its high sensitivity, precision, reproducibility, and ability to separate complex mixtures effectively [1]. RP-HPLC is extensively applied for qualitative and quantitative estimation of drugs in bulk materials, pharmaceutical dosage forms, and biological samples.

Fexofenadine Hydrochloride is a second-generation antihistamine drug commonly prescribed for the treatment of allergic rhinitis and chronic urticaria. It acts by selectively blocking peripheral histamine H1 receptors and produces minimal sedative effects compared to first-generation antihistamines [2]. Due to its widespread therapeutic use, the development of sensitive, accurate, and robust analytical methods for the estimation of Fexofenadine Hydrochloride is essential for routine quality control and regulatory compliance.

Several analytical techniques such as UV spectroscopy, HPLC, LC-MS, and spectrofluorimetry have been reported for the determination of Fexofenadine Hydrochloride. However, RP-HPLC remains the preferred technique because it offers better resolution, shorter analysis time, high accuracy, and improved sensitivity [3]. The efficiency of RP-HPLC

methods depends upon several chromatographic parameters including mobile phase composition, pH, column selection, flow rate, and detection wavelength. Optimization of these parameters using conventional trial-and-error methods is often time-consuming and may not provide robust analytical performance [4].

To overcome these limitations, the Analytical Quality by Design (AQbD) approach has gained significant importance in modern pharmaceutical analysis. AQbD provides a systematic and scientific method for analytical method development by understanding the relationship between critical method variables and analytical responses [5]. Among the different experimental design techniques, Central Composite Design (CCD) is widely employed for method optimization because it evaluates the interaction effects between variables and helps in achieving optimum chromatographic conditions with fewer experimental runs [6].

II. PHARMACEUTICAL ANALYSIS

The substance will be in any dose form and may consist of a single ingredient or a combination of compounds. Animals, plants, microorganisms, minerals, and different synthetic items are all employed as medications.

Titration, precipitation, spectroscopy, chromatography, and other methods are frequently employed in this area of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the measurement of the proportions of the constituents (quantitative analysis).

Analytical chemistry is defined as the study of materials by breaking them down into their constituent parts and determining the quantity of each.

Types

There are primarily two types of qualitative analysis.

1.Qualitative (identifying)

2. Quantitative (approximation)

The composition of natural and manufactured substances is determined by qualitative investigation. These tests are carried out to determine whether or not the compound or substance is present in the sample. Detection of evolved gas, precipitate formation, limit tests, color change reactions, freezing point and boiling point tests, and more are examples of qualitative testing.

2. The primary purpose of quantitative analytical procedures is to measure any component or compound present in the sample. These methods are based on (a) the quantitative execution of an appropriate chemical reaction and either measuring the amount of reagent added to finish the reaction or measuring the amount of reaction product acquired, and (b) the characteristic movement of a substance.

through a specified medium in a controlled environment, (c) electrical measurement, and (d) assessment of the compound's spectroscopic characteristics.[7]

Liquid Chromatography with High Performance: The basic idea is to inject a little amount of sample at or near the head of a stainless-steel tube (column) filled with microscopic particles into the flowing liquid (mobile phase). The mobile phase, which is supplied by a pump and forced through the column by high pressure at a constant speed, subsequently carries the sample's component components down the column.

Crucial elements of HPLC

- Superior resolution
- Glass column, stainless steel, small diameter

Quick analysis

- A comparatively elevated mobile phase pressure
- Measured mobile phase flow rate [8]

III. DRUG PROFILE OF FEXOFENADINE HYDROCHLORIDE

Compared to first-generation antihistamines, fexofenadine has minimal side effects since it is a highly selective H1-receptor antagonist with little affinity for cholinergic or α-adrenergic receptors. Furthermore, fexofenadine does not cause drowsiness and does not affect focus, memory, or performance due to its lack of CNS penetration. Fexofenadine is quickly absorbed in adults, reaching peak plasma concentrations one hour after delivery. A single 130 mg dose achieves maximum histamine inhibition one to two hours after administration. Similarly, it has been demonstrated that giving children 30 mg or 60 mg of fexofenadine suppresses histamine-induced wheal and flare within 1 to 2 hours. [9]

IUPAC Name

The chemical designation of Fexofenadine Hydrochloride according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature is:

(±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α,α-dimethyl benzeneacetic acid hydrochloride [10].

The chemical framework of the drug includes aromatic rings, hydroxyl groups, a piperidine moiety, and a carboxylic acid functional group. These structural features contribute significantly to the physicochemical characteristics of the compound, including polarity, solubility behavior, and chromatographic retention properties. Such molecular attributes are important considerations during RP-HPLC method development and optimization [11].

Molecular Formula and Molecular Weight

The molecular formula of Fexofenadine Hydrochloride is:

$C_{32}H_{39}NO_4 \cdot HCl$

The compound possesses a molecular weight of 538.12 g/mol [1].

The analytical behavior of Fexofenadine during chromatographic separation is influenced by its molecular size and amphoteric nature. Parameters such as ionization tendency, hydrophobic interaction, and molecular polarity affect chromatographic responses including retention time, peak shape, and separation efficiency. Therefore, understanding molecular characteristics is essential for selecting suitable chromatographic conditions in RP-HPLC analysis [12].

Drug Category

Fexofenadine Hydrochloride belongs to the pharmacological category of second-generation non-sedating antihistamines. It functions by competitively blocking peripheral H1-histamine receptors, thereby suppressing histamine-mediated allergic manifestations such as itching, sneezing, rhinorrhea, and skin wheals [2].

Due to poor penetration into the central nervous system, the drug produces substantially lower sedation compared with conventional first-generation antihistaminic agents. This favorable pharmacological profile has increased its clinical acceptance in allergy management [13].

Mechanism of Action

By blocking peripheral histamine H1 receptors, fexofenadine hydrochloride, a second-generation selective H1-antihistamine, lessens allergy symptoms as itching, vasodilation, edema, and inflammation. By restricting histamine-mediated reactions and maintaining the inactive state of the H1 receptor, it functions as an inverse agonist. It causes little sedation because of its limited blood–brain barrier penetration. By lowering inflammatory mediators implicated in allergic responses, fexofenadine also demonstrates anti-inflammatory efficacy.[14]

Fexofenadine Hydrochloride's Analytical Significance

Since fexofenadine hydrochloride is a commonly used antihistaminic medication recommended for allergic illnesses, precise analytical determination is required to preserve pharmaceutical quality, safety, and therapeutic efficacy. Assay determination, formulation analysis, stability studies, impurity monitoring, and regular quality control testing all depend on analytical evaluation of fexofenadine. Many analytical methods have been used to estimate it, but RP-HPLC is widely used due to its excellent precision, sensitivity, selectivity, and repeatability in pharmaceutical analysis.[15]

Immune Component	Modified Action of Fexofenadine
H1 Histamine Receptor	Acts through competitive blockade of H1 receptors
Leukotrienes (LTC4, LTD4, LTE4)	Suppresses leukotriene synthesis
Prostaglandins (PGE2, PGF2α)	Reduces production of inflammatory prostaglandins
Cyclo-oxygenase-2 (COX-2)	Demonstrates inhibitory activity against COX-2 enzyme
Thromboxane	Lowers thromboxane generation
iNOS mRNA	Decreases inducible nitric oxide synthase expression
ELAM-1	Down-regulates adhesion molecule production
VCAM-1	Reduces vascular adhesion molecule expression
Tryptase	Minimizes release of inflammatory mediator tryptase
Nasal Epithelial Cells	Decreases eosinophil interaction and reduces release of IL-6 and TNF-α
Mucosal Fibroblasts	Suppresses secretion of matrix metalloproteinases (MMP-2 and MMP-9)

Eosinophils	Reduces release of inflammatory mediators including RANTES, IL-8, GM-CSF, and sICAM-1
Keratinocytes	Lowers expression of inflammatory chemokines such as RANTES, I-TAC, MDC, and TARC
Peripheral Blood Leukocytes	Decreases IL-4 production

Pharmacokinetic Profile

The maximal plasma concentration (T_{max}) of fexofenadine hydrochloride is typically reached 1-3 hours after dosing due to its quick oral absorption. The medication supports once- or twice-daily dosage regimes because it exhibits dose-dependent pharmacokinetics across the therapeutic range and has an elimination half-life of roughly 11–15 hours. Fexofenadine is mostly removed via biliary and renal excretion, with little hepatic metabolism. Its absorption and disposal are greatly influenced by drug transporters like organic anion transporting polypeptides (OATPs) and P-glycoprotein (P-gp). Drug interactions, genetic polymorphisms, and medical conditions can all have an impact on the pharmacokinetic characteristics of fexofenadine, which can impact systemic drug exposure and clearance.[16]

Analytical Importance of Fexofenadine Hydrochloride

One clinically significant second-generation antihistaminic medication that is frequently used to treat allergic diseases is fexofenadine hydrochloride. The development of precise and trustworthy analytical techniques is crucial to guaranteeing the quality, safety, efficacy, and consistency of pharmaceutical formulations due to its wide range of pharmaceutical applications. Fexofenadine hydrochloride must be analytically evaluated for drug assay, method validation, stability studies, impurity assessment, and routine quality control analysis.

Among available analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is extensively employed because of its superior sensitivity, specificity, precision, and reproducibility. RP-HPLC facilitates effective chromatographic separation with acceptable retention behavior, symmetrical peak characteristics, and short analysis time. Validated RP-HPLC methods are capable of evaluating critical analytical parameters including linearity, accuracy, precision, robustness, specificity, limit of detection (LOD), and limit of quantification (LOQ). These analytical attributes establish RP-HPLC as a reliable and efficient technique for quantitative estimation of Fexofenadine Hydrochloride in pharmaceutical dosage forms and routine laboratory investigations.[17]

Development of the RP-HPLC Method

Using ideal chromatographic conditions, an RP-HPLC technique was created for the simultaneous measurement of Montelukast and Fexofenadine Hydrochloride. In order to achieve efficient analyte separation and satisfactory chromatographic performance, method development required choosing the appropriate mobile phase composition, detection wavelength, and flow rate. A methanol: phosphate buffer (75:25 v/v) mobile phase with a flow rate of 0.8 mL/min and UV detection at 215 nm was used in the optimized procedure. In these circumstances, effective separation was achieved with retention durations of 6.50 min for Fexofenadine and 3.02 min for Montelukast. For routine pharmaceutical analysis, the optimized chromatographic method yielded acceptable peak symmetry, sufficient resolution, and appropriate analytical sensitivity.[18]

A. Selection of the Stationary Phase

Analyte retention, separation efficiency, and peak resolution are all greatly impacted by the choice of stationary phase, making it a crucial stage in the development of RP-HPLC methods. Fexofenadine hydrochloride was analyzed using a Cap Cell Pack C18 column (250 × 4.6 mm, 5 µm) as the stationary phase in the manner described. Good peak symmetry, excellent chromatographic performance, and efficient analyte separation from the internal standard were all given by the C18 reverse-phase column. Effective interaction with the analyte was made possible by the stationary phase's non-polarity, which produced adequate retention characteristics appropriate for standard pharmaceutical analysis.[19]

B. Optimization of the Mobile Phase

Because mobile phase optimization directly impacts retention time, peak symmetry, resolution, and analytical sensitivity, it is an essential stage in the development of RP-HPLC methods. To achieve an acceptable chromatographic separation for

fexofenadine hydrochloride, several ratios of acetonitrile and buffer systems were assessed. Mobile phase composition, pH, and flow rate were among the parameters that were methodically optimized. The mobile phase used in the optimized chromatographic condition was Acetonitrile: Buffer (35:65 v/v) with triethylamine, adjusted to pH 2.0. This resulted in improved peak shape, acceptable retention behavior, and dependable chromatographic performance for the quantitative analysis of fexofenadine hydrochloride.[20]

C. Optimization of Flow Rate

Because flow rate adjustment directly affects retention duration, peak shape, resolution, and total analysis time, it is a crucial stage in the development of RP-HPLC methods. In order to achieve good chromatographic separation, various flow rates are typically assessed during technique optimization for fexofenadine hydrochloride. It was discovered that an effective separation with a suitable retention duration, satisfactory peak symmetry, and an enhanced theoretical plate count could be achieved at a flow rate of 0.8 mL/min. Chromatographic performance was not significantly affected by small intentional changes in flow rate (±0.1 mL/min), demonstrating the robustness of the established approach. In regular pharmaceutical analysis, appropriate flow rate selection also helps to improve technique repeatability and reduce solvent usage.[21]

Choice of Detection Wavelength

Using a UV-visible spectrophotometer, a standard solution was scanned throughout the 200–400 nm wavelength range to determine the detection wavelength for fexofenadine hydrochloride. For the RP-HPLC analysis, the wavelength at which maximum absorbance was measured (λ_{max}) was selected as the detection wavelength. Fexofenadine hydrochloride's λmax was determined to be 220 nm. For the duration of the investigation, 220 nm was chosen as the detecting wavelength.[22]

Preparing Samples

Twenty tablets or capsules containing 10 mg of fexofenadine hydrochloride were precisely weighed and powdered in order to prepare the sample for the analysis in pharmaceutical dosage forms (tablets/capsules). To achieve the required concentration, the powder was moved to a volumetric flask, dissolved in mobile phase, sonicated for ten to fifteen minutes, filtered through a 0.45 µm membrane filter, and appropriately diluted.[23]

Validation of Methods

The following parameters were used to validate the proposed RP-HPLC technique for the estimation of fexofenadine hydrochloride in accordance with ICH Q2(R2) guidelines:

Specificity

By proving that the analyte peak was clearly separated from excipients, contaminants, and degradation products, specificity was established. The method's stability-indicating nature was verified using forced degradation investigations (acid, base, oxidative, thermal, photolytic, and hydrolytic).

linearity

Standard solutions were prepared at various concentration levels in order to assess linearity. Plotting the calibration curve between peak area and concentration revealed a correlation coefficient (r2) greater than 0.999, showing satisfactory linearity within the given range.[24]

Range

The linearity, accuracy, and precision evaluations determined the analytical procedure's range. It encompasses the range where the method exhibits adequate linearity, precision, and accuracy between the higher and lower concentrations.

Precision

Recovery experiments were used to determine accuracy. The sample matrix was spiked with known concentrations of fexofenadine hydrochloride. The degree of similarity between the measured and true values was used to compute the percentage recovery.[25]

Robustness

During the method's development, robustness was assessed by purposefully making little adjustments to crucial parameters like flow rate, mobile phase composition, pH, and column temperature. The method's dependability under regular use was confirmed by the fact that it remained unchanged.

System Suitability Testing

To guarantee the chromatographic system's performance before sample analysis, system suitability parameters were set and tracked.

The developed and verified RP-HPLC technique is appropriate for routine quality control of fexofenadine hydrochloride in pharmaceutical dosage forms and satisfies all pertinent performance parameters as per ICH Q2(R2) recommendations.[26]

Analytical Quality by Design (AQbD) and Central Composite Design (CCD)

In order to obtain dependable and repeatable analytical performance, analytical quality by design, or AQbD, is a systematic methodology used in the creation of analytical methods. To guarantee constant method quality, the strategy relies on defining predetermined analytical objectives, comprehending crucial method variables, and adjusting experimental settings. AQbD enhances method robustness, regulatory acceptability, and process comprehension while supporting methodical analytical development.[27]

Within the AQbD paradigm, Central Composite Design (CCD) is a crucial statistical tool for analytical method optimization. It makes it possible to examine several chromatographic variables at once, including mobile phase composition, pH, flow rate, and detection wavelength. It also assesses how these variables affect analytical responses, such as retention time, peak symmetry, resolution, and peak area. Using CCD reduces the number of experimental runs, finds the impacts of variable interactions, and helps achieve ideal chromatographic conditions with increased analytical robustness and efficiency.[28]

Design of Experiments

Design of Experiments (DoE) is a statistical methodology that examines how various variables affect experimental responses in order to systematically refine analytical and pharmacological methods. Factorial design, Box-Behnken design, and Central Composite Design (CCD) are common DoE techniques. DoE helps create RP-HPLC methods by assessing variables including flow rate, pH, and mobile phase composition to achieve reliable and optimal analytical performance.[29]

CCD or Central Composite Design

A statistical optimization tool called Central Composite Design (CCD) is used in the development of RP-HPLC methods to examine the effects of several analytical variables at once. In order to increase chromatographic performance, including retention time, peak area, and resolution, it assists in optimizing variables including mobile phase composition, pH, flow rate, and injection volume. CCD reduces the number of experimental trials and helps create solid and trustworthy analytical techniques.[30]

A sophisticated experimental design technique used during analytical optimization to comprehend how particular method variables affect chromatographic results is called Central Composite Design (CCD). CCD makes it possible to systematically examine crucial operating variables in RP-HPLC experiments, which helps researchers anticipate and improve method behavior. The design facilitates the enhancement of analytical features including chromatographic efficiency, peak profile, and separation quality by evaluating the combined impact of variables like mobile phase conditions and instrumental settings. The development of dependable analytical techniques is aided by the use of CCD, which encourages effective optimization with less laboratory testing [31].

IV.CONCLUSION

The significance of RP-HPLC method development and validation for fexofenadine hydrochloride in pharmaceutical analysis is emphasized in this review. Reliable analytical techniques are necessary to guarantee the identity, purity, potency, and quality of fexofenadine hydrochloride, a second-generation antihistaminic drug that is frequently given, in pharmaceutical formulations. Because of its superior accuracy, sensitivity, specificity, precision, and reproducibility, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has become a preferred method among the current analytical techniques.

Careful adjustment of crucial chromatographic parameters, such as stationary phase choice, mobile phase composition, pH, flow rate, detection wavelength, and system appropriateness conditions, is necessary for the successful development of RP-HPLC methods.

Prospects for the Future

Future studies on the development and validation of RP-HPLC methods for fexofenadine hydrochloride are probably going to concentrate on the broader application of Central Composite Design (CCD) for systematic analytical optimization and Analytical Quality by Design (AQbD) principles. Analytical techniques that are more robust, dependable, and repeatable can be produced by using CCD to better understand crucial chromatographic factors and the consequences of their interactions.

A greater focus on green analytical chemistry could promote the adoption of less expensive chromatographic techniques, safer solvents for the environment, and less solvent use in RP-HPLC analysis. Automation, chemometric tools, and computational modeling could also improve data interpretation, method dependability, and analytical optimization.

In order to help pharmaceutical quality control, formulation development, and regulatory needs, future research may further investigate the development of stability-indicating, impurity profiling, and bioanalytical RP-HPLC methods for fexofenadine hydrochloride. It is anticipated that the advancement of contemporary analytical method development for pharmaceutical applications will be greatly aided by the combination of AQbD principles with CCD-based optimization.

REFERENCES

[1] L. R. Snyder, J. J. Kirkland, and J. W. Dolan, *Introduction to Modern Liquid Chromatography*, 3rd ed. New York: John Wiley & Sons, 2010.

[2] L. L. Brunton, R. Hilal-Dandan, and B. C. Knollmann, *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 13th ed. New York: McGraw-Hill Education, 2018.

[3] E. Akbel and I. Bulduk, "RP-HPLC method development and validation for quantification of fexofenadine in pharmaceutical products," *European Journal of Science and Technology*, 2021.

[4] H. M. Maher, M. A. Sultan, and I. V. Olah, "Development of validated stability-indicating chromatographic method for the determination of fexofenadine hydrochloride and its related impurities in pharmaceutical tablets," *Chemistry Central Journal*, vol. 5, 2011.

[5] International Council for Harmonisation (ICH), *ICH Q8 (R2): Pharmaceutical Development*. Geneva, Switzerland: ICH, 2009.

[6] D. C. Montgomery, *Design and Analysis of Experiments*, 8th ed. New York: John Wiley & Sons, 2013.

[7] Zheng C, *Introduction to Pharmaceutical Analysis for Drugs*, Research & Reviews: Journal of Pharmaceutical Analysis (RRJPA), 2021; 10(5): 1–2.

[8] A. M. Girase, B. M. Mahale, A. R. Dhankani, and S. P. Pawar, "Review On: RP-HPLC," *Journal of Advances in Experimental Therapeutics and Neurotherapeutics*, vol. 2, no. 2, pp. 21-27, 2024.

[9] E. O. Meltzer, N. A. Rosario, H. Van Bever, and L. Lucio, "Fexofenadine: review of safety, efficacy and unmet needs in children with allergic rhinitis," *Allergy, Asthma & Clinical Immunology*, vol. 17, article 113, 2021.

[10] Indian Pharmacopoeia Commission, *Indian Pharmacopoeia*, Vol. II. Ghaziabad, India: IPC, 2022.

[11] Y. Kazakevich and R. Lobrutto, *HPLC for Pharmaceutical Scientists*. New Jersey: Wiley-Interscience, 2007.

[12] L. R. Snyder, J. J. Kirkland, and J. W. Dolan, *Introduction to Modern Liquid Chromatography*, 3rd ed. New York: John Wiley & Sons, 2010.

[13]. B. G. Katzung, *Basic and Clinical Pharmacology*, 15th ed. New York: McGraw-Hill Education, 2021.

[14] M. Batool, A. Zamir, F. Alqahtani, T. Ahmad, H. Saeed and M.F. Rasool, "Clinical Pharmacokinetics of Fexofenadine: A Systematic Review," *Pharmaceutics*, vol. 16, no. 1619, 2024.

[15] P. Arefin, et al., "Review on Fexofenadine: Safety, efficacy and therapeutic applications," *International Journal of Applied Pharmaceutics*, vol. 14, no. 1, pp. 13-17, 2022

[16] M. A. Alvi, *et al*., "Clinical Pharmacokinetics, Drug Interactions and Transporter Pharmacogenetics of Fexofenadine: A Comprehensive Review," *Pharmaceutics*, vol. 16, no. 12, Article 1619, 2024.

[17] Akbel and I. Bulduk, "RP-HPLC Method Development and Validation for Quantification of Fexofenadine in Pharmaceutical Products,"

European Journal of Science and Technology, no. 32, pp. 1048-1053, 2021.

[18] S. A. Ahmed and V. P. Shinde, "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Montelukast and Fexofenadine in Pharmaceutical Formulation," *Journal of Pharma Insights and Research*, vol. 3, no. 3, pp. 326-332, 2025

[19] N. Malothu, *et al*., "Development and Validation of RP-HPLC Method for Estimation of Fexofenadine Hydrochloride Using Levocetirizine as Internal Standard," *International Journal of Pharmacy and Biological Sciences*, vol. 8, no. 3, pp. 619-625, 2018.

[20] S. A. Ahmed, *et al*., "RP-HPLC Method Development and Validation for Simultaneous Determination of Paracetamol and Fexofenadine Hydrochloride," *Acta Chromatographica*, vol. 37, no. 3, pp. 337-343, 2025.

[21] Buchupalli P, Medidi S. *RP-HPLC Method for the Simultaneous Estimation of Ambroxol Hydrochloride and Fexofenadine Hydrochloride in Bulk and in a Tablet Mixture*. J Appl Pharm Sci. 2015;5(02):074-080.

[22] M. Vimal raj and M. Sumithra, "Analysis of second-generation anti histamine fexofenadine soft gelatin capsules and its related compound by using RP-HPLC," Ann. Phytomed., vol. 12, no. 1, pp. 616-627, 2023.

[23] M. Vimal raj and M. Sumithra, "Analysis of second-generation anti histamine fexofenadine soft gelatin capsules and its related compound by using RP-HPLC," Ann. Phytomed., vol. 12, no. 1, pp. 616-627, 2023.

[24] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, "Validation of Analytical Procedures Q2(R2)," ICH Harmonised Guideline, November 2023.

[25] International Conference on Harmonisation, "Validation of Analytical Procedures: Text and Methodology Q2(R1)," ICH Harmonised Tripartite Guideline, November 2005.

[26] International Council for Harmonisation, "Validation of Analytical Procedures Q2(R2)," ICH Harmonised Guideline, Step 2b, 24 March 2022.

[27] P. D. Ikhar, P. G. Gawande, P. D. Thakre, P. D. Darange, and V. D. Dapurkar, "A Review on Analytical Method Validation as Per ICH Guidelines and Protocols," *International Journal of Pharmaceutical Sciences*, vol. 3, no. 11, pp. 2595-2600, 2025.

[28] International Council for Harmonisation (ICH), *ICH Q14: Analytical Procedure Development*, Geneva, Switzerland, 2023.

[29] S. N. Politis and D. M. Rekkas, "Design of experiments (DoE) in pharmaceutical development," *Drug Development and Industrial Pharmacy*, 2017.

[30] A. Gupta, S. P. Rachana, and S. Moorkoth, "Central Composite Design Aided Optimization and Validation of Developed an Eco-Friendly HPLC Method for the Quantification of Lenalidomide Loaded Mesoporous Silica Nanoparticles," *Journal of Applied Pharmaceutical Science*, vol. 15, no. 01, pp. 089-101, 2025.

[31] A. Najmi, *et al*., "AQbD-based Central Composite Design approach for HPLC method optimization and validation," *Journal of Saudi Chemical Society*, vol. 28, 2024, Article 101772.