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Review article

Chromatographic Separation of Antihistamine Drugs using HPLC

Shafat Ahmad Khan[*,1], *Syed Dilshad Alam*[2], *Muralidhararao Bagadi*[3], *Rushda Anjum*[4], *Mohd Yusuf*[*, 5]

[1,4]*Department of Chemistry, School of Basic Sciences, Galgotias University, Greater Noida, U.P. 201310 India*
[2,3]*Analytical Research & Development, Jubilant Biosys Limited, Knowledge Park- II, Greater Noida, Uttar Pradesh 201310 India*
[5]*Glocal University, Mirzapur Pole, Saharanpur, Uttar Pradesh 247121 India*

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ABSTRACT

The chromatographic separation of antihistamine drugs using High-Performance Liquid Chromatography (HPLC) has garnered significant attention due to its pivotal role in pharmaceutical analysis. Antihistamines, vital in managing allergic disorders, present complex challenges in terms of analytical assessment owing to their diverse chemical structures and stereochemistry. This review aims to provide an overview of one of the best methodologies employed in HPLC-based separation of antihistamine drugs. Emphasis is placed on the separation of both nonracemic and racemic forms, considering their distinct pharmacological profiles and therapeutic implications. Various aspects, including method development, optimization strategies, column selection, and detection techniques, are scrutinized to highlight the crucial parameters influencing separation efficiency and accuracy. Furthermore, the review delves into the application of chiral HPLC methods for resolving enantiomeric mixtures of antihistamines, underscoring their importance in pharmaceutical research and development. By synthesizing recent literature findings and methodological advancements, this review offers insights into the current state-of-the-art in HPLC-based separation of antihistamine drugs and outlines future directions for research in this critical field.

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1. Introduction

Antihistamines, essential pharmaceuticals in combating allergic reactions, work by blocking histamine receptors in the body, thereby alleviating symptoms such as nasal congestion, sneezing, and hives triggered by allergens like pollen, dust mites, or animal dander. However, the effective extraction and identification of antihistamine drugs present a considerable challenge due to their diverse chemical structures and stereochemistry. High-Performance Liquid Chromatography (HPLC) has emerged as a leading analytical technique for this purpose, offering high resolution, sensitivity, and reproducibility in separating and quantifying individual compounds within complex mixtures (Yusuf et al., 2023; Asif et al., 2024). Anti-histamines are a family of pharmaceuticals that block the action of histamine receptors in the body. The term "antihistamine" is normally used by common people for a medication-switch can cure allergies; however, medical professionals and researchers use it in a specialized sense as a specialized kind of over-the-counter medicine that inhibits some of the effects of histamines.

Our body develops an allergy when its immune system has an exaggerated response to "foreign or external" chemicals. Dust and animal dander, both of which are often considered to be innocuous and aren't a problem for some people, might become a problem for other people if they have an allergy to them. These compounds are interpreted as compounds "foreign," due to which the self-defence mechanism of their body overreacts. This overreaction also involves the production of histamine. When histamine is released in excess it can create a variety of problems. Congestion, coughing, wheezing, and shortness of breath are some of the symptoms that may be brought on by an excess of histamine (Fig. 1 and Fig. 2) (Ali et al. 2023; da Costa et al. 2019; Lim and Lord 2002; Kang et al. 2010). This excess is brought on by our body's hypersensitivity and exaggerated reaction to an allergen. Other symptoms of excess histamine include exhaustion or fatigue; itchiness in the different areas of the skin, including hives and various skin rashes; itching is felt in the eyes and they become red, and water starts coming out of the eyes; sneezing; a runny or stuffy nose, or both; insomnia.

The success of HPLC-based analysis hinges upon the strategic selection of chromatographic columns, which significantly influence separation efficiency and selectivity. Reversed-phase columns, commonly used in antihistamine analysis, exploit differences in hydrophobicity to separate

* ***Corresponding Author***:
Email: shafat5000[a]gmail.com (SA Khan) & yusuf1020[a]gmail.com (M. Yusuf)
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compounds, while normal-phase columns, though less differences (Simon and Simons 2008; Ali et al. 2023). Chiral columns, specialized for resolving enantiomeric mixtures, are particularly valuable for separating the distinct pharmacological properties of racemic antihistamine formulations, ensuring both safety and efficacy in prevalent, offer complementary selectivity based on polarity pharmaceutical development. Optimization of chromatographic conditions and careful selection of detection techniques further enhance the efficacy of HPLC analysis in antihistamine drug separation and identification.

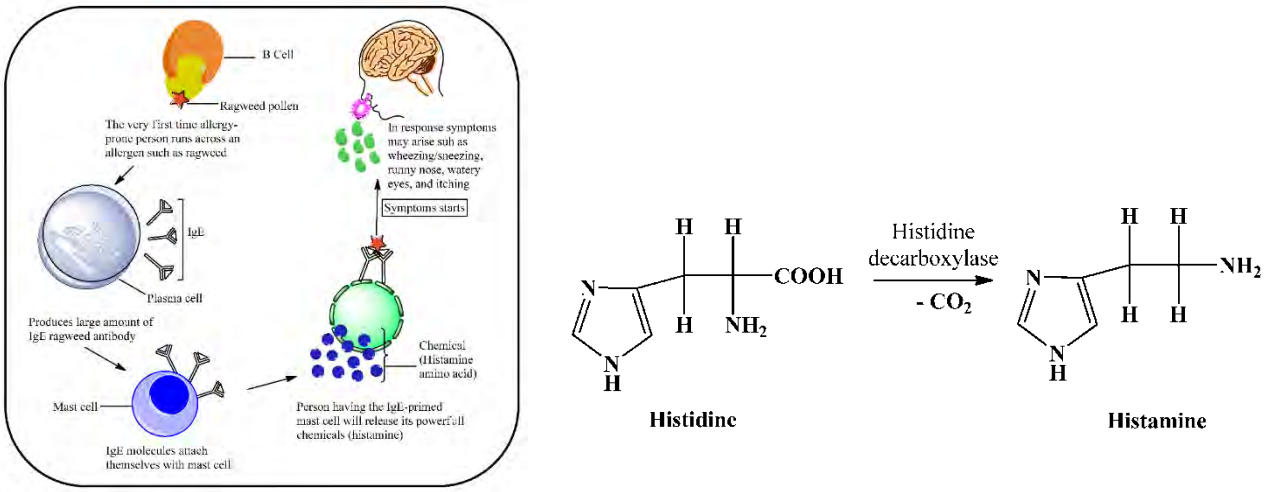
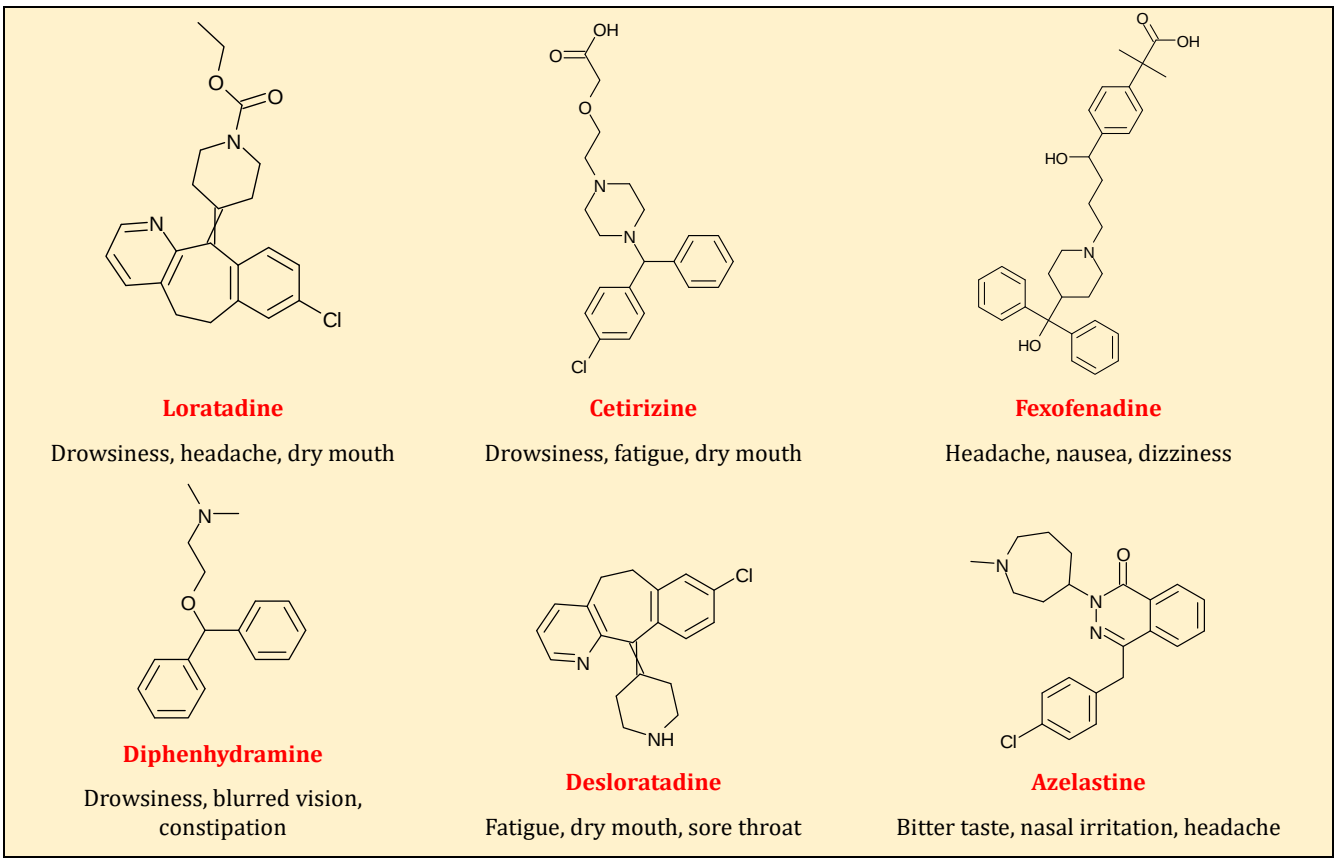


Fig. 1. Schematic illustration of excessive immune response of the general allergic pathway (Adapted from Ali et al. under CCBY MDPI 2023).

Method development involves systematic optimization of parameters such as mobile phase composition, pH, flow rate, and temperature to achieve desired analytical goals, while detection methods like UV-V is spectroscopy, fluorescence detection, and mass spectrometry enable precise quantification and identification of separated compounds. Through continued advancements in HPLC methodology, researchers aim to deepen our understanding of antihistamine pharmacology and contribute to the development of safer and more efficacious allergy medications.



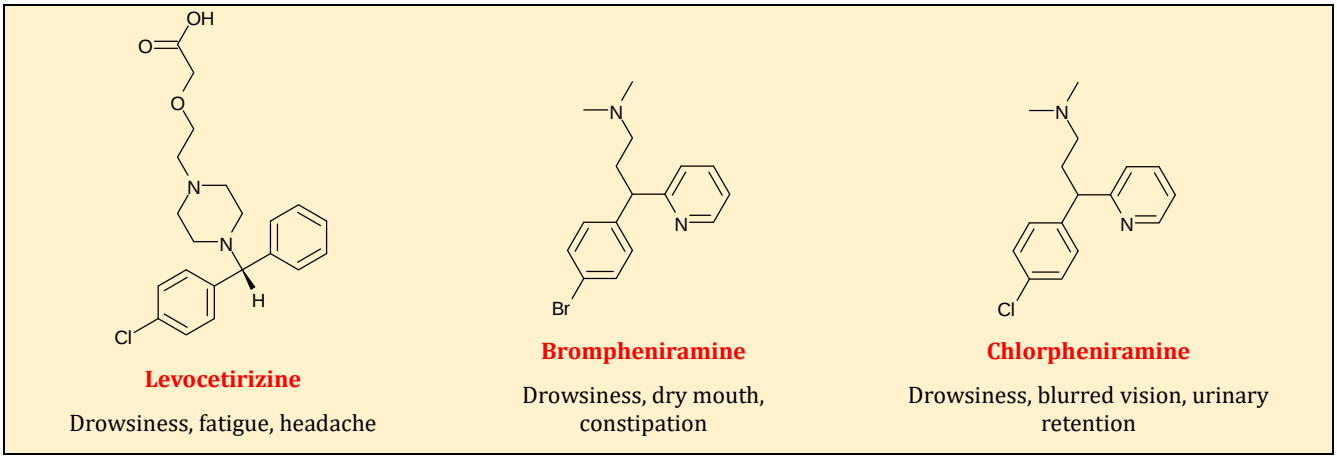


Fig. 2. Some antihistamine drugs with their associated Adverse Health Responses.

2. Methods for Separation of Antihistamines

The separation of antihistamine drugs is a crucial aspect of pharmaceutical analysis, considering the widespread prevalence of allergic conditions and the necessity for effective treatment options. Various analytical techniques have been explored to achieve efficient separation, among which capillary electrophoresis (CE), supercritical fluid chromatography (SFC), and high-performance liquid chromatography (HPLC) stand out as particularly promising methods. Each of these techniques offers unique advantages and capabilities in separating antihistamine compounds, contributing to the comprehensive understanding of their pharmacokinetics and pharmacodynamics.

Capillary electrophoresis (CE) has gained prominence as a powerful tool for separating antihistamine drugs due to its high separation efficiency, short analysis time, and minimal sample consumption. CE operates on the principle of differential migration of charged analytes through a capillary tube under the influence of an electric field. This technique offers excellent resolution for enantiomeric separations, making it particularly suitable for studying chiral antihistamine compounds. Studies have extensively explored the enantio-separation of antihistamines such as dioxo promethazine, doxylamine, hydroxyzine, and cetirizine using CE, providing valuable insights into their stereochemical properties and pharmacological activities.

Supercritical fluid chromatography (SFC) represents another promising approach for separating antihistamine drugs, leveraging the unique properties of supercritical fluids as the mobile phase. SFC employs carbon dioxide (CO_2) as the primary solvent, which is maintained above its critical temperature and pressure, resulting in a supercritical state characterized by both liquid-like and gas-like properties. This technique offers advantages such as rapid analysis, environmental friendliness, and compatibility with a wide range of analytes, including polar and nonpolar compounds. While SFC has been less extensively explored for antihistamine separations compared to HPLC and CE, ongoing research efforts continue to uncover its potential in this domain, particularly in resolving challenging compounds and enantiomeric mixtures.

High-performance liquid chromatography (HPLC) remains the cornerstone technique for antihistamine separation, owing to its versatility, robustness, and widespread availability in pharmaceutical laboratories. HPLC operates by passing a liquid mobile phase through a stationary phase within a chromatographic column, with analytes being separated based on their differential interactions with the stationary phase. This technique offers excellent resolution, sensitivity, and reproducibility, making it well-suited for analyzing complex mixtures of antihistamine compounds. Studies have extensively investigated the separation of various antihistamines using HPLC, focusing on parameters such as column selection, mobile phase composition, and detection techniques to optimize separation efficiency and accuracy. In recent years, considerable research attention has been directed towards the enantio-separation of antihistamine drugs using HPLC, particularly with chiral stationary phases. Enantiomers, or mirror-image forms of a molecule, often exhibit differences in pharmacological activity, metabolism, and toxicity, highlighting the importance of separating them for pharmacological studies and drug development. HPLC with chiral columns offers a powerful approach for achieving this separation, enabling the characterization of individual enantiomers and elucidation of their stereochemical properties. Studies have explored the enantioselective separation of antihistamines such as cetirizine, loratadine, and fexofenadine using chiral HPLC columns, providing valuable insights into their pharmacokinetics and pharmacodynamics. Nevertheless, the separation of antihistamine drugs using analytical techniques such as CE, SFC, and HPLC plays a crucial role in pharmaceutical research and development. These techniques offer complementary advantages in terms of separation efficiency, selectivity, and sensitivity, allowing for the comprehensive characterization of antihistamine compounds. Continued advancements in analytical methodologies, coupled with ongoing research efforts, are poised to further enhance our understanding of antihistamine pharmacology and contribute to the development of safer and more efficacious allergy medications.

Studies have been conducted on an extensive scale on the enantio-separation of dioxo promethazine, doxylamine, hydroxyzine, and cetirizine, by using capillary electrophoresis (CE). The enantiomers of cetirizine were separated by Hu et al. (2002) using HPLC with a chiral ovomucoid column as the stationary phase. Chiral stationary phases (CSPs) of proteins, on the other hand, have a propensity to be less stable. By using LC–MS, Kang et al. (2010) were able to extract cetirizine on a Chiralpak AD-H column. Zhou et al. (2016) developed a method for the chiral separation of the enantiomers of six different antihistamines, which include doxylamine, carbinoxamine, dioxo promethazine, oxomemazine, and cetirizine, using a stereoselective high-performance liquid chromatography technique. They also looked at how the retention duration and resolution were affected by the mobile phase additive, the temperature of the column, and the flow rate. To determine the amounts of four antihistamine drugs—diphenhydramine, chlorpheniramine, cyproheptadine, and fexofenadine—in pharmaceutical forms, an improved and basic ion-pair HPLC technique was devised by Shasho et al. (2018).

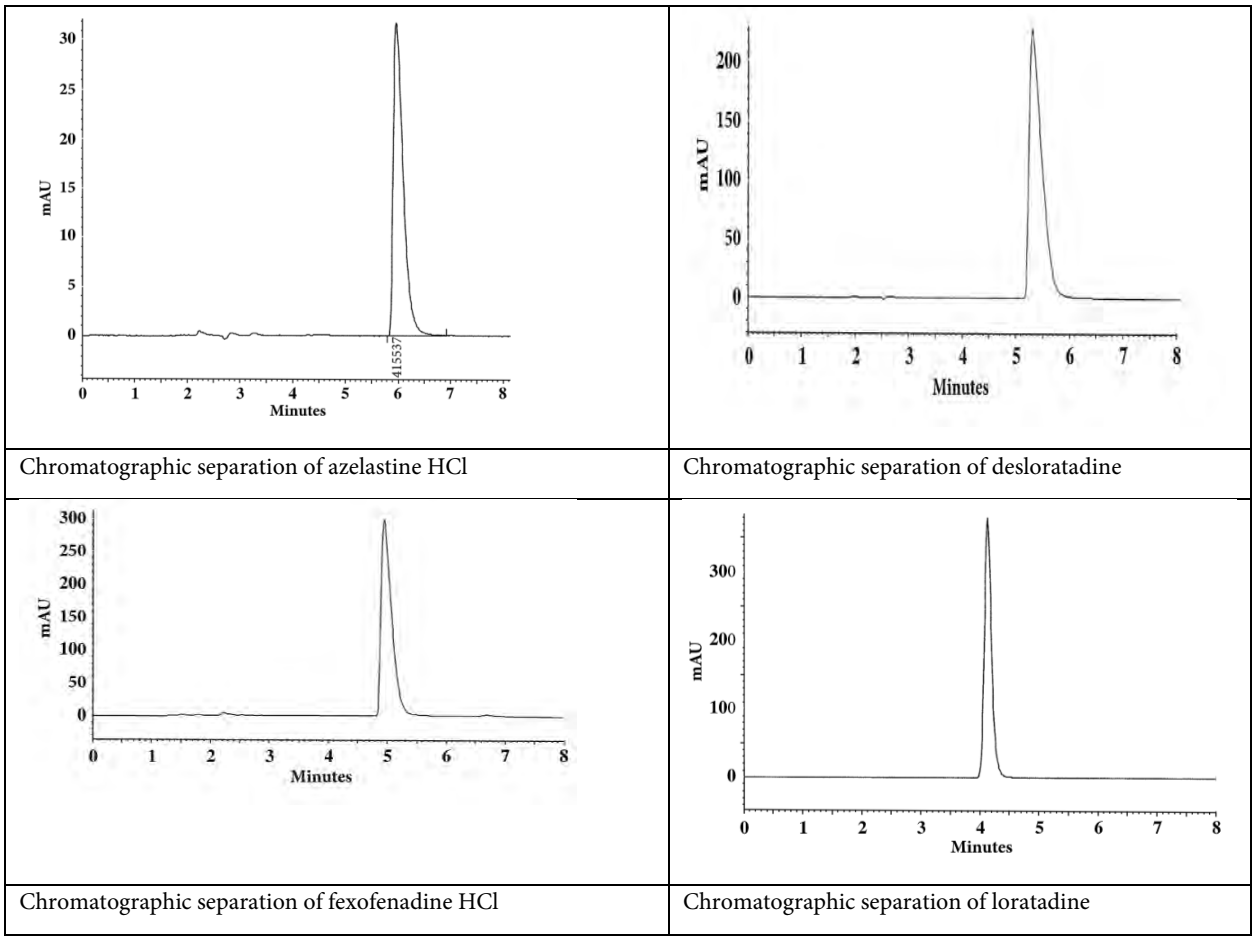


Fig. 3. Chromatograms of some studied antihistamine drugs using Chromatographic separation technique (Adapted from da Costa et al. under CCBY Hindawi 2019).

This approach proved to be easy and accurate. To perform chiral separations of pheniramine, oxybutynin, cetirizine, and brinzolamide in human plasma, an environmentally friendly, repeatable, accurate, cost-effective, and high-performance SPE-Chiral-HPLC technique was developed by Ali et al. (2021). They carried out the % recoveries calculations at a range of pHs, including 5.0, 7.0, and 9.0 respectively. Pheniramine, oxybutynin, cetirizine, and brinzolamide each were found to have maximum percentage recoveries of 32.50 and 37.67, 34.61 and 37.33, 22.48 and 23.59, and 33.60 and 44.80, respectively, when the pH was set to 9.0. Xiong et al. (2021) established a convenient and reliable normal-phase chiral HPLC technique for enantiomeric separation and quantitative analysis of S- and R-HCQ. They made the quantitative determination of the amounts of hydroxychloroquine enantiomers possible by the baseline separation of two enantiomers. Methods developed by them were all proven for accuracy, precision, sensitivity, specificity, and repeatability in the initial round of testing. Further, the method developed by them can be used to quantify hydroxychloroquine enantiomers for the chiral switch as well as for pharmacokinetic and toxico-kinetic studies, which could be useful for thoroughly assessing and comparing the efficacy and toxicity profiles of hydroxychloroquine enantiomers (Ali et al. 2014).

3. High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography or HPLC is a kind of column chromatography which is more advanced than conventional column chromatography. In normal chromatography solvent flows down the column on its own due to force of gravity acting on it but in the HPLC, an additional high pressure of up to 400 atm is employed so that the solvent can move down the column rapidly (Tao et al., 2007). One more advantage of HPLC is that it allows us to use particles which are much smaller than those of normal chromatography, as the packing material of the columns. Due to the smaller size of the particles, the surface area for the interaction between the mobile phase and the stationary phase increases. As the interaction increased, as a result, it was able to segregate different components of the mixture in a much more effective and efficient way. HPLC technique has more degree of sensitivity, and it also involves a great level of automation. HPLC is now excessively used couple with LC-MS for the separation of antihistamines due to its low cost and reliability. Method involves following steps: (a) Sample preparation, (b) Selection of chromatographic conditions, and (c) Optimization.

3.1 Sample Preparation

In their study of separation and assay of four antihistamine drugs diphenhydramine, chlorpheniramine, Cyproheptadine and Fexofenadine Shasho et al. (2018) took twenty tablets of different anti-histamines taken from the market. They were crushed in a mortar and pestle to convert them into powdered form. Then an amount of this powder which contains approximately twenty-five milligrams of antihistamine was taken and it was then transferred to a thousand-milliliter volumetric flask. This volumetric flask also has deionized water in it. The content of the volumetric flask was diffused by putting it under the influence of a magnetic stirrer for about 20 minutes. The solution was then taken and it was solicited for about ten minutes. The procedure is done for 10 minutes as it was long enough to dissolve all the pharmaceutical ingredients very well. In their study of the separation of Pheniramine, Oxybutynin, Cetirizine, and

Brinzolamide (Ali et al., 2014; Ali et al., 2023; Xiong et al. 2021), obtained working standards from various vendors on the market. In separate containers filled with methanol, solutions of these pharmaceuticals with concentrations of 1.0, 0.5, and 0.1 mgmL-1 were made, respectively. In their separation of antihistamines, Zhou et al. (2016) dissolved Doxylamine, carbinoxamine, oxomemazine, dioxo promethazine, hydroxyzine and cetirizine in suitable volumes of ethanol. Filtration of solutions of each of these was done so that their sample solution could be prepared.

3.2 Selection of chromatographic conditions

For Zhou et al. (2016) as the primary component of the mobile phase, n-hexane served as the solvent of choice. Ethanol or isopropanol was selected to play the role of mobile phase modifier, and DEA was used as the mobile phase additive. After being ultrasonically degassed and filtered through a 0.45-m solvent filter, the mobile phase was ready for use. The cetirizine, carbinoxamine, oxomemazine, hydroxyzine, and dioxo promethazine detection wavelengths were all set to 227 nm, while the doxylamine detection wavelength was set at 262 nm. 10 microliters was the amount of sample that was injected (Ali et al., 2021).

3.3 Optimization

Ali et al. (2014) in order to find the optimal conditions for the creation of samples followed the procedure as follows: 1.0 milliliters of pheniramine, 1.0 milliliters of oxybutynin, 1.0 milliliters of cetirizine, and 1.0 milliliters of brinzolamide were individually combined with 5.0 milliliters of fresh-frozen human plasma. The human plasma samples that had been spiked were vortexed for two minutes and then stored for thirty. After that, 15.0 mL of acetone was added to each sample that had been vortexed, and the mixture was maintained for 30 minutes. In order to isolate the supernatant, these samples were centrifuged for ten minutes at a speed of 10,000 revolutions per minute (11,180 g). After removing all of the moisture from the supernatant using a vacuum, it was redissolved in 10.0 mL of phosphate buffer (50 mM, pH 7.0). Preconditioning was accomplished by adding 2.0 mL of methanol and 5.0 mL of Millipore water to 1.0 mL cartridges. After that, a flow rate of 0.1 mLmin-1 was used to move 10.0 mL of phosphate buffer (50 mM, pH 7.0) containing pheniramine, oxybutynin, cetirizine, and brinzolamide through the cartridge. This was followed by cleaning the cartridges with 2.0 mL of Millipore water while maintaining the same flow rate. The cartridges were then dried using hot air, and the reported medicines were eluted using 10.0 milliliters of methanol with 0.1% trifluoroacetic acid at a flow rate of 0.1 milliliters per minute. Under a vacuum, the eluted methanol solutions for each of the listed medicines were concentrated to a volume of 0.5 milliliters. Chiral-HPLC analyses were then carried out using these samples as input. A number of different experimental parameters were modified in order to improve the solid phase extraction conditions (Raja et al., 2021; Ali et al., 2022).

4. Conclusion

The examination of chromatographic separation techniques applied to antihistamine drugs utilizing High-Performance Liquid Chromatography (HPLC) underscores the indispensable nature of this analytical methodology within the field of pharmaceutical research and development. HPLC emerges as the preeminent choice owing to its inherent versatility, exceptional sensitivity, and unwavering reliability in discerning even the most minuscule concentrations of drug compounds. As our comprehension of HPLC methodologies continues to evolve and refine, we edge closer towards the realization of safer and more efficacious treatments for allergic disorders. This review stands as a cornerstone reference for forthcoming investigations aimed at optimizing chromatographic techniques tailored to the precise analysis of antihistamine drugs, thereby fostering tangible benefits for patients on a global scale.

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Conflict of Interests

The authors declare that they have no conflict of interest and approved for final submission.

Authors Contributions

SA Khan: Concept, design, first-draft, data collection, interpretation, analysis, Communication; ***SD Alam:*** Design, edit-draft, data interpretation; ***M Bagadi:*** Data interpretation, edit-draft, analysis; ***R Anjum:*** Design, edit-draft, data collection, analysis and ***M Yusuf:*** Concept, design, edit-draft, data interpretation, analysis, Communication.

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