STM JOURNALS

Research & Reviews: A Journal of
Drug Formulation, Development and Production

ISSN: 2394-1944
Volume 11, Issue 1, 2024
January–April
DOI (Journal): 10.37591/RRJoDFDP

https://journals.stmjournals.com/rrjodfdp

Review RRJoDFDP

Advancements in Method Development and Validation for Levocetirizine: A Comprehensive Review

Patil Divyashree Kantilal[1,*], Amit Kumar R. Dhankani[2], Mansi A. Dhankani[3], S.P. Pawar[4]

Abstract

This review work delves into the intricate realm of concurrent estimation of multiple compounds, with a specific emphasis on Levocetirizine and its related substances. It looks into how to improve analytical process specificity by using reverse phase high-performance liquid chromatography (RP-HPLC) in conjunction with UV detection. The study meticulously explores the intricacies of method development and validation, offering invaluable insights into the realms of precision and accuracy within pharmaceutical analysis. Throughout the discourse, the review sheds light on the nuanced methodologies employed, illustrating their efficacy in concurrently estimating multiple compounds. The emphasis on Levocetirizine and its related substances underscores the relevance and applicability of these findings within pharmaceutical contexts. By scrutinizing the method development and validation processes, the review not only highlights the technical nuances involved but also provides a comprehensive understanding of the underlying principles governing precision and accuracy in analytical procedures. The significance of the findings reverberates within the realm of pharmaceutical analysis, as they underscore the pivotal role of advanced RP-HPLC techniques. Through meticulous experimentation and analysis, the review elucidates the potential of these techniques to enhance specificity, thereby augmenting the reliability and robustness of pharmaceutical analyses. Furthermore, the insights gleaned from the study offer a roadmap for researchers and practitioners seeking to navigate the complexities of concurrent compound estimation. In essence, this review work serves as a cornerstone in the field of pharmaceutical analysis, offering a comprehensive exploration of RP-HPLC methodologies for concurrent compound estimation. Its conclusions not only make a substantial contribution to the corpus of current knowledge, but they also highlight how important accuracy and precision are in pharmacological analyses. As such, it provides a seminal reference point for further research and development endeavors within this burgeoning field.

***Author for Correspondence**
Patil Divyashree Kantilal
E-mail: divyashree2609@gmail.com

[1]Student, Department of Pharmaceutical Quality Assurance, Poojya Sane Guruji Vidya Prasarak Mandal's College of Pharmacy, Shahada, Maharashtra, India
[2]Assistant Professor, Department of Pharmaceutical Quality Assurance, Poojya Sane Guruji Vidya Prasarak Mandal's College of Pharmacy, Shahada, Maharashtra, India.
[3]Assistant Professor, Department of Pharmaceutics, Poojya Sane Guruji Vidya Prasarak Mandal's College of Pharmacy, Shahada, Maharashtra, India
[4]Principal, Poojya Sane Guruji Vidya Prasarak Mandal's College of Pharmacy, Shahada Maharashtra, India

Received Date: March 14, 2024
Accepted Date: March 27, 2024
Published Date: April 12, 2024

Citation: Patil Divyashree Kantilal, Amit Kumar R. Dhankani, Mansi A. Dhankani, S.P. Pawar. Advancements in Method Development and Validation for Levocetirizine: A Comprehensive Review. Research & Reviews: A Journal of Drug Formulation, Development and Production. 2024; 11(1): 15–27p.

Keywords: RP-HPLC, pharmaceutical analysis, Levocetirizine, UV detection, method development

INTRODUCTION

The techniques used in analytical chemistry include material identification, separation, and quantification for both natural and synthetic compounds. Many factors influence the choice of analytical methodology, including the number of samples, the speed and cost of the analysis, the chemical characteristics of the analyte and its concentration, sample matrix, and the type of measurements (qualitative or quantitative). One can

use a qualitative method to ascertain the species' chemical identity in the sample. Regarding the relative concentrations of one or more analytes in the sample, a quantitative approach offers numerical data. There are more and more new medications coming onto the market each year. These medications could be brand-new creations or a partial structural alteration of already-existing ones.

The date a medicine is first introduced to the market and the date it is included in pharmacopoeias frequently coincide. This occurs as a result of the potential risks associated with continuing to use these medications more widely, reports of new toxicities that prompt their removal from the market, the emergence of patient resistance, and the launch of more effective medications by rival companies. The pharmacopoeias might not have the standards and analytical techniques for these medications under these circumstances. Therefore, the development of newer analytical techniques for such medications becomes important [1].

Chemical substances can be found, recognized, described, and quantified using analytical chemistry methods. In biology, this approach is widely used for both pharmaceutical product quality monitoring and research development. There are two types of analytical methods in analytical chemistry: (1) classical methods and (2) current methods. The wet chemical method is the traditional approach; the legislative method is the modern approach. There are two main categories for the classical analytical method: (1) Classical qualitative analytical method; and (2) Classical quantitative analytical method. These two types can be further distinguished by some recent analytical techniques: (1) Modern qualitative analytical techniques; and (2) Modern quantitative analytical techniques.

1. *The classical technique:* The majority of the principles used in modern equipment and the foundations of analytical methods may be traced back to classical approaches. Sophisticated instrumentation dominates analytical chemistry. This method, which is still widely used today, is also the cornerstone of most instructional laboratories for undergraduate analytical chemistry courses. This is a basic analytical chemistry technique.
2. *Qualitative analysis:* Due to the lack of quantification, qualitative analysis can only indicate the existence or absence of a component-not its mass or concentration. Determining the chemical makeup of a sample is known as qualitative analysis in chemistry.
3. *Chemical tests:* The acid test for gold and the Kastle-Mayer test for the presence of blood are two examples of qualitative chemical tests that are accessible. A basic chemical test can be used to identify a number of important ingredients in food. While some tests quantify a compound's concentration, others only show its presence in food.
4. *Flame test:* Through a series of reactions to rule out other scenarios, inorganic analysis is a scientific technique that verifies the existence of a particular equation or element. Assumed ions are then confirmed through a confirming test. With present equipment, small carbon-containing ions are occasionally included in such schemes; these tests are infrequent but will be useful for fieldwork, teaching, and other scenarios where access to cutting-edge instruments is restricted or nonexistent.
5. *Quantitative analysis:* The determination of how much of one or more elements are present in the sample, which may also consist of solid gases or a mixture, is the focus of quantitative analysis. To determine the quantity of a particular analyte in an analytical sample, the traditional quantitative analytical approach looks for changes in mass and volume. Gravimetric analysis and volumetric analysis both are analysis methods [2].

UV Spectrophotometry

Ultraviolet-visible (UV-Vis) spectroscopy is widely used to analyse and characterise a wide range of materials. Organic molecules and functional groups, as well as inorganic and organic solid or liquid groups, can be found using UV-visible spectroscopy. Moreover, reflectance for coatings, paints, textiles, biochemical studies, dissolution kinetics, band gap measurements, and other uses can be measured using it. Based on the variations in sample responses and the degree of transmission or absorption of a distinct beam light wavelength, UV-Vis provides these details.

Beer's Law

An absorbing substance will cause the incident radiation intensity (Io) to be higher than the emerging radiation intensity when an electromagnetic radiation beam passes through it. Beer's law is a generic law that can be used to quantitatively quantify the radiant energy absorption by materials.

Beer's law states that the length of the radiation's transit through the sample, b (cm), and the amount of the absorbing substance present, c (moles/l), are inversely related to the amount of radiation that is transmitted or absorbed (absorbance, A) by a solution or medium. Consequently, a straight line with a slope of Єb should pass through the origin when plotting absorbance versus concentration.

$A = -\log(I/Io) = Єbc$

where $Є = k/2.303$

Concentration and path length have no effect on the molar absorptivity, which is a constant.

An organic molecule's concentration can be found using Beer's law equation by locating its maximum absorbance in the UV-Vis absorption spectrum, provided that the route length and molar absorptivity are known.

Principle of UV-Vis spectroscopy

When radiation alters an ion's or molecule's electrical structure, an object will exhibit absorption in the visible or ultraviolet spectrum. Because of this, a sample's molecules undergo an electronic state change when they absorb light in the ultraviolet or visible spectrum. Electrons in their ground-state orbital will be pushed by the light's energy into an excited-state orbital, which has a higher energy or orbital against bonding. Three different kinds of ground-state orbitals could be involved [3].

High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is one of the most effective analytical chemistry tools available today. This method allows for the separation, identification, and quantification of any component present in a sample that is soluble in a liquid. Drug product analysis, both quantitative and qualitative, commonly uses the most accurate analytical method, HPLC. The basic notion is that a sample solution is put into a stationary phase composed of porous material, and then a liquid (the mobile phase) is pumped through the column at high pressure. Sample separation is based on the difference in migration rates across the column that arises from an uneven distribution of the sample's stationary and mobile phases. Elution occurs at different times based on how various components behave during partitioning. In contrast to compounds with less affinity, which move quicker and farther, the sample compound with a higher affinity for the stationary layer will move slower and cover a shorter distance. HPLC is a more flexible technology than gas chromatography since it may be employed with a larger variety of mobile and stationary phases and is not limited to volatile and thermally stable substances.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

The mobile phase in RP-HPLC is either polar or somewhat polar, while the stationary phase is non-polar. The foundation of RP-HPLC is hydrophobic interaction. Analytes that are comparatively less polar in a mixture of components will be retained by the non-polar stationary phase for a longer amount of time than those that are noticeably more polar. As a result, the most polar component will elute initially [4].

Validation

In the US, the concept of validation was developed in 1978. The concept of validation has grown over time to include a wide range of activities, including analytical methods for guaranteeing the quality of medicinal substances and medical goods, as well as computerized systems for clinical trials, process control, or labelling. Validation is most effectively understood as a crucial part of CGMP. The

evaluation of validity, or the process of proving efficacy, is called validation. Validation is a team endeavour involving people from several plant specialties. The "process of establishing documented evidence", sometimes referred to as "method validation", provides a high level of assurance regarding the suitability of the product or equipment for the intended analytical applications.

Procedures for Validating a Method

1. Create a validation master plan, an operational process, or a validation methodology.
2. Describe the method's uses, goals, and extent.
3. Specify the dimensions of performance and the requirements for acceptance.
4. Explain what validation tests include.
5. Confirm the equipment's related performance parameters.
6. Quality-check materials, such as reagents and standards.
7. Carry out trial preparation for validation.
8. If necessary, modify the method's parameters or acceptance standards.
9. Carry out comprehensive validation experiments, both internal and external.
10. Create SOPs to include the procedure in regular operations.
11. Establish the revalidation criteria.
12. Specify the kind and frequency of analytical quality control (AQC) checks and/or system suitability tests for the routine.
13. Record validation trials and findings for the validation.

Parameters (Components) of Method Validation

1. Accuracy.
2. Precision.
3. Linearity.
4. Limit of detection.
5. Limit of quantitation.
6. Specificity.
7. Range.
8. Robustness.
9. Ruggedness [5].

METHOD OF DEVELOPMENT OF LEVOCETIRIZINE

UV Spectrophotometric Method

Farhana Lipi et.al, (2023) has developed the method: Levocetirizine Dihydrochloride is dissolved in a 0.1 M hydrochloric acid solution, and the resulting solution is then measured using UV spectroscopy according to the recommended procedure. The tests were done at a wavelength of 231 nm, which was found to be the absorption maximum. In the concentration range of 7.5 to 22.5 μg/ml, Beer's law was adhered to the calibration curve's linearity between concentration and absorbance, was demonstrated by the line equation $y=0.0377x–0.0043$ ($R^2=0.9992$) repeatability of procedures since the percentage RSD was determined to be less than 2%. In this instance, the suggested methodology was robustly, specifically, linearly, accurately, and precisely validated statistically in compliance with ICH guidelines. The results were judged to have very satisfactory accuracy and precision. Levocetirizine dihydrochloride routine analysis benefits from the method's simplicity, specificity, speed, reproducibility, accuracy, and economy, as demonstrated by validation studies [6].

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) Method

1. Gupta *et al*. [7]: In the discipline of pharmaceutical chemistry, the development and validation of analytical methods is an important research tool [7]. The drug content in mixes and pharmaceutical formulations can be ascertained using these techniques. The literature and industrial scenario demonstrate the effectiveness of analytical instruments such as hyphenated chromatography and different chromatographic techniques for the quantification of drugs in

formulations, even at low concentrations, by using HPLC techniques. Before a pharmaceutical dosage form is released onto the market, the department of quality assurance and quality control must determine the purity of the active components. Nowadays, a patient's compliance with therapy is greatly influenced by the potency and efficacy of the medications. Nonetheless, a number of analytical techniques have been developed in recent decades for the examination of medications in terms of quality assurance and control. Pharmaceutical chemistry is advancing quickly, and sensitive chromatographic and spectral techniques can be used for the quantification and analysis of active analytes in pharmaceuticals. In the pharmaceutical industry, the analyst is crucial to the FDA's approval of new, potent drugs because they help with method development, validation, and drug determination. The validation parameters, such as selectivity (matrix interference), sensitivity, linearity, precision and accuracy batches (minimum three), matrix effect, recovery, ruggedness, stock solution stability, reinjection stability, long-term stability, and dilution integrity, were proven for specific drugs. Analytical methods must be straightforward, sensitive, selective, rugged, and reproducible. This study's primary objective was chosen in response to the pharmaceutical industry's growing requirement for appropriate analytical procedures.

Chromatographic stipulations:

i. The mobile phase's organic phase ratio needs to be adjusted by an absolute ±2%. (i.e., triethylamine, methanol, purified water (69:19:12) for 2%, and Mobile Phase A as a whole.
ii. Colum temperature is to be changed to ±5°C (i.e., 35 and 45°C)
iii. *Column:* 4.6 mm×25 cm; 5 µm packing L3.
iv. Flow rate to be changed by ±10% (i.e., 0.9 and 1.1 ml/min).
v. Mobile phase a pH to be changed by ±0.2 units (i.e., pH 2.3 and pH 2.7).
vi. *Wavelength:* 231 nm.
vii. *Injection volume:* 20 µl [7].

2. *Rao et al.* [8]: the Reversed phase chromatography was used to perform HPLC separation on an XTerra C8 (4.6×150 mm, 3.5 µm) analytical column that was kept at room temperature. Acetonitrile (60:40 v/v) in phosphate buffer (pH 4) was utilized as the mobile phase. The flow rate at which it was run was 0.8 ml/min and detected UV light at 230 nm. The Levocetirizine HCl and Montelukast retention times under optimal circumstances are 2.432 and 6.218 min, respectively. For both medications, the technique was found to be linear over an analytical range of 30–70 µg/ml. The Levocetirizine HCl and Montelukast LOD and LOQ values are 3.36 and 9.90 and 3.20 and 9.86 µg/ml, respectively. The suggested method's high precision and accuracy were demonstrated by the low percentage RSD values (<1) and outstanding recovery values, respectively. The approach was demonstrated to be robust and rugged by the % RSD values for factors such as method robustness and method ruggedness. The Levocetirizine HCl and Montelukast sodium simultaneous determination in combination tablet dosage form was accomplished with success using the described method. For Levocetirizine, the recovery percentage was 99.1%, while for Montelukast, it was 98.0%. There was no indication that the co-formulated chemicals were interfering. Therefore, Levocetirizine hydrochloride and Montelukast sodium measurement in combined tablet dose form may benefit from and be appropriate for the suggested technique.

Chromatographic parameters

i. *Equipment:* High performance liquid chromatography equipped with Auto Sampler and DAD (Dual Absorbance Detector) detector.
ii. *Column:* Symmetry C8 (4.6×150 mm, 3.5 µm, Make: XTerra).
iii. *Flow rate:* 0.8 ml/min.
iv. *Wavelength:* 230 nm.
v. *Injection volume:* 20 µl.
vi. *Column oven:* Ambient.
vii. *Column oven:* Ambient.
viii. *Run time:* 8 min [8].

Reverse Phase Ultra Performance Liquid Chromatography (RP-UPLC) Method

Shetgar et al. [9]: a Waters Acquity UPLC with BEH, a C18 column measuring 100 mm×2.1 mm, a 1.7 μm column at 0.3 ml/min flow rate and an Acquity TUV detector at 230 nm were used to accomplish this work. This procedure was validated using the ICH recommendations as a foundation. A 0.999 R^2 rating indicated linearity at 25 to 150% of the data. With a mean recovery of 99.02%, precision and accuracy were within the range recommended by the ICH. The sensitivity of the RP-UPLC method is 0.04 μg/ml for the Limit of Quantitation (LOQ) and 0.04 μg/ml for the Limit of Detection (LOD). Studies on degradation under various conditions (oxidation, acidity, base, temperature, water, UV light) show that neither placebo nor degradation contaminants affect the primary peak of Levocetirizine. Its straightforward, sensitive, accurate, quick, and stability-indicating approach makes it a useful tool for regular quality control testing of formulations and the active pharmaceutical ingredient.

Chromatographic conditions: The Waters Acquity UPLC chromatographic system was employed, with a BEH C18 column of 100 mm×2.1 mm, or 1.7 μm. The mobile phase was composed of 60:40 (pH 4.80) acetonitrile and 0.01 N potassium dihydrogen phosphate (KH_2PO_4) buffer. The injection volume was 2 μl, the flow rate was set at 0.3 ml/min, and the column oven was set at 30°C. Acetonitrile and 0.01 N KH_2PO_4 buffer were mixed 50:50 to create the diluent. Using an Acquity-Tunable UV detector set at 230 nm, detection was accomplished. The instrument operation, data processing, and data gathering were all done with Empower 2 software (Table 1) [9].

RP-HPLC Method for Simultaneous Estimation

1. *Butala et al.* [10]: the medications were separated by chromatography using a stationary phase of a 250×4.6 mm, 5 μm Hypersil ODS C18 column. A mixture of methanol, acetonitrile, and 20 mm ammonium acetate buffer was used as the mobile phase. The ratio was 60:30:10 v/v. The flow rate was set at 0.8 ml/min, and the column temperature was set at 35°C to detect at 232 nm. It took the three drugs around 10 min to elute fully. System appropriateness, selectivity, linearity, precision, accuracy, limits of detection, and limits of quantification were assessed in accordance with ICH requirements in order to validate the developed method. With assay results of 99.89% w/w for LTZ, 100.30% w/w for ABP, and 100.59% w/w for MTKT, the method demonstrated accuracy. It was also precise, with intra-day and inter-day percentage RSDs less than 2%, and robust, with percentage RSD less than 2% for the three medications. The discovered technique can be used to measure LTZ, ABP, and MTKT-containing fixed-dose combination products simultaneously [10].
2. *Kumar et al.* [11]: A phenomenex C18 column (150×4.6 mm i.d., particle size of 5 μ) was used for the separation process, which involved using a combination of 0.01 M potassium dihydrogen orthophosphate (pH 5.0±0.05) and acetonitrile (60:40 v/v) as the mobile phase in an isocratic elution mode at a flow rate of 1 ml/min. At 230 nm, the detection was observed. Levocetirizine's retention time was determined to be around 3.60 min, while Ambroxol's was found to be 4.68 min. For Ambroxol, an excellent linearity range was reported between 12 and 120 μg/ml, and for Levocetirizine, 1–10 μg/ml. The technique was successfully used to determine Levocetirizine and Ambroxol simultaneously from the combination dosage formulation. It was validated in terms of linearity, robustness, precision, and accuracy.

Table 1. Optimized chromatographic conditions [11].

Parameters	Method
Stationary phase (Column)	Phenomenex C18 (150×4.6 mm i.d, 5 μ size)
Mobile phase	0.01 M Potassium dihydrogen orthophosphate (pH 5.0±0.05) and Acetonitrile (60:40 v/v)
Flow rate (ml/min)	1
Pressure (kgf)	194
Run time (min)	10
Column temperature (°C)	Ambient
Detection wavelength (nm)	230
Drugs retention time (min)	3.60 min (Levocetirizin) 4.68 min (Ambroxol)

3. *Ali et al.* [12]: the separation of 3 µm was obtained on Hypersil C18, measuring 100×4.6 mm. The mobile phase is a blend of acetonitrile and buffer at a 90:10 v/v ratio. The buffer is made by dissolving 2.8 gm of disodium hydrogen orthophosphate in 1000 ml of filtered water, then adding diluted orthophosphoric acid to get the pH level up to 7.0. The mobile phase had a flow rate of 1.0 ml/min, and the entire elution period lasted 15 min. The studies were carried out at 25°C using a 230 nm UV detection wavelength. In terms of selectivity, linearity, precision, accuracy, limits of detection, and quantification for the contaminants in accordance with ICH criteria, the developed approach was successfully applied to the determination of the medications under examination in tablets [12].
4. *Rani et al.* [13]: the Hypersil BDS 250×4.6 mm, 5 µ was used to run the chromatogram. A mobile phase consisting of 35% buffer and 65% acetonitrile was pumped through the column at a flow rate of 1 ml/min. The buffer utilised in this procedure was a pH 3.6, 0.02 M Potassium dihydrogen phosphate buffer. A constant temperature of 30°C was maintained. The ideal wavelength for Levocetirizine and Montelukast was 231 nm. Levocetirizine and Montelukast were shown to have retention times of 2.599 and 3.472 min, respectively. The Levocetirizine and Montelukast RSDs were discovered to be 0.7 and 0.8, respectively. For Levocetirizine and Montelukast, the percentage recovery was 99.89 and 99.88%, respectively.
   Instrumentation and chromatographic conditions: A high-performance liquid chromatography system comprising a Waters 2695 with a 2996-module Photo Diode Array detector, an automatic sample injector, a quaternary solvent delivery pump, and a column thermostat was used to complete the analysis. Empower2 software was used for both the data collection and processing. The chromatographic separation was carried out using a 250 mm×4.6 mm×5 µ Hypersil BDS column. The flow rate they kept constant was 1 ml/min. The column's temperature was consistently maintained at 30°C. An appropriate retention time and good resolution between Montelukast and Levocetirizine were obtained using a mobile phase consisting of a 35:65 ratio of potassium dihydrogen phosphate buffer and acetonitrile. At 231 nm, the procedure was optimized. The empower2 system software was used for both data processing and collection. A 7-min run time was recorded. At room temperature, all of the calculations are made [13].
5. *Basu et al.* [14]: by using an isocratic elution mode and a mixture of 0.05 M potassium dihydrogen phosphate buffer (pH 7.5) and methanol (20:80 v/v) as the mobile phase, the method was developed using a Waters HPLC system on a L7 column (Hypersil Gold: 250 mm×4.6 mm, 5 µm) at 35°C and a load of 10 µl. At 225 nm, the detection was done. Levocetirizine and Montelukast were shown to have retention times of about 3.2 and 4.2 min, respectively. Levocetirizine HCl and Montelukast Sodium were quantitatively determined from the tablet dosage form simultaneously using the validated method, which was found to be linear, robust, precise, and accurate.
   Instrumentation: The analysis was conducted using an Isocratic Waters HPLC equipped with a 515 pump, a 2487 dual-wavelength UV-visible detector, and an L7 column (Hypersil Gold: 250 mm×4.6 mm, 5 µm). Empower 2 software was a useful addition to the HPLC system's data processing capabilities.

Stability Indicating by RP-HPLC Method

Sonawane et al. [15]: Waters 2695 separation module with a PDA detector was used to construct the suggested RP-HPLC method. A column called Hypersil BDS C18 (250/4.6 mm, 5 µm) was used for chromatographic separation, which was carried out at a flow rate of 1 ml/min for duration of 10 min. The mobile phase consisted of acetonitrile and a 40:60% v/v phosphate buffer. The pH was adjusted to 7.0 with orthophosphoric acid, and a PDA detector was used to scan the eluents at 230 nm. LEV and MON had retention durations of 3.06 and 6.76 min, respectively. For LEV and MON, a linearity response was noted within the dose range of 12.56–37.68 and 23.78–71.20 µg/ml, respectively. For LEV, the limits of detection and quantification are 0.079 and 0.239 µg/ml, respectively, and for MON, they are 0.156 and 0.473 µg/ml. In order to develop the stability-indicating method, drugs were exposed to stress conditions such as acid, base hydrolysis, oxidation, neutral, photo-, and thermal degradation. The degraded products that resulted from the samples were successfully solved.

Stability-indicating, LC-MS compatible method and Assay by UHPLC

Sai Kumar et al. (2018): a brand-new gradient RP-UHPLC technique has been created and approved for use in the assessment of Levocetirizine HCl and Montelukast sodium in tablets containing 2.5 and 4 mg of the respective drugs. Using a Kinetex Phenyl-Hexyl (100°A, 150×4.6 mm, 5 µm) column kept at 20±2°C, the two medications were satisfactorily separated from their associated contaminants. A flow rate of 1.2 ml/min was the ideal value. A fixed wavelength of 230 nm and an injection volume of 20 µl were used. As part of the method's appropriateness assessment, tests for sensitivity, linearity, relative response factor establishment, quantitation limit, and forced degradation were conducted. In order technique was verified in mass analysis of both pharmaceuticals and all contaminants using UHPLC parameters with mass parameters. The identical column, flow rate, and injection volume were used in the development of the assay technique. Here, however, the column oven temperature was 30±2°C, resulting in a shorter runtime. The validation process included determining the standard, sample, and mobile phases' specificity, accuracy, linearity, precision, and solution stability. The assay method's filter variability was assessed by calculating the similarity factor between the unfiltered and PVDF assay data using nylon.

Instrument and chromatographic condition in UHPLC for Related Substances (Optimized method): Agilent (U-HPLC) (Model: 1260 Infinity II & DAD) was utilized in conjunction with the Empower 3.0 data integrator module. An analytical column from Kinetex, Phenyl-Hexyl (100°A, 150 mm×4.6 mm, 5 µ) kept at 20±2°C was used to perform the separation. At 230 nm, the wavelength was optimal. 1.2 ml/min flow rate and 20 µl injection volume, were set. The needle wash was 90% methanol in water. This gradient algorithm was done by proportionally mixing two distinct mobile phases. Trifluoroacetic acid and water were mixed in a single mobile phase at a 1000:1.5 ratio. Another mobile phase consisted of a 5100:900 combination of acetonitrile and methanol, to which 9 ml of trifluoroacetic acid were added. Both mobile phases were initially combined in a 70:30 ratio. The ratio shifted to 60:40 at 15 min, then to 50:50 at 28 min. At min-40, the ratio shifted once more to 30:70. At 45 min, the ratio changed to 20:80. The ratio was restored to 70:30 at min-50, and remained there for an additional 55 min. Therefore, it lasted for 55 min (Tables 2 and 3).

Instrument and chromatographic condition in LC-MS (Optimized method): In this case, the U-HPLC portion was conducted using the developed related substance approach. For the purpose of characterizing

Table 2. Chromatographic conditions [14].

Parameters	Conditions
Column	Hypersil Gold L7 (4.6×250 mm, 5 µm)
Mobile phase	0.05 M KH_2PO_4 Buffer pH 7.5: Methanol 20:80 v/v
Flow rate	1.2 ml/min
Temperature	35°C
Detection wavelength	225 nm
Injection volume	10 µ
Diluent	Mobile phase

Table 3. Optimized conditions of chromatography [15].

Parameters	Conditions
Column	Hypersil BDS C18, 250 mm×4.6 mm, 5 µm
Mobile phase	*Acetonitrile:* Phosphate buffer pH-7.0 (60:40)
Flow rate	1.0 ml/min
Column temperature	30°C
Injection volume	10 µ
Detection wavelength	230 nm
Run time	10 min
Retention time	3.06 (LEV), 6.76 (Montelukast) min

the Mass Lynx 4.1 data integrator module, Waters Micromass (Model: Quattro micro API, Alliance 2695) was utilized. A full scan was chosen under the mass acquisition approach. In the mass range of 50 to 800 amu, measurements were made for both polarities (+ve and -ve). A 30 V cone voltage was the ideal setting [16].

Bioanalytical Method and Estimation of Levocetirizine in Blood plasma by using RP-HPLC

Khatri et al. [17]: the medication was extracted from plasma using an optimised liquid-liquid extraction process. Acetonitrile, methanol, and 20 mm ammonium acetate buffer pH-5 (25:55:20% v/v/v) were used as the mobile phase in the analysis of the sample. Chromatographic separation was carried out with a Thermo C-18 column (4.6×250 mm, 5 µ particle size) as the stationary phase, employing isocratic elution and a flow rate of 1 ml/min. The chromatographic separation took 8 min to complete, and the peak was found using a UV-PDA detector set at 232 nm. Within the concentration range of 2–10 µg/ml, a linear calibration curve (r^2=0.9998) was found. The accuracy, resilience, and recovery of the method were confirmed. The limits of quantitation and detection were 0.174 µg/ml and 0.0057, respectively. The amount of drug spiked in plasma did not significantly differ from the amount collected, and plasma did not cause any problems throughout the estimating process. As a result, the suggested technique works well for analysing LD in human plasma and tablet dose forms.

Chromatographic conditions: For the chromatographic analysis, a mobile phase comprising 20 mm ammonium acetate buffer (pH 5.0), methanol, and acetonitrile (20:55:25 v/v) was employed. Prior to use, these were sonicated to remove any remaining gas and filtered using a 0.45 µ membrane filter. Isomerically pumped at 1.0 ml/min, the mobile phase was subjected to analysis at room temperature. On a Thermo C-18 column (4.6×250 mm, 5 µ particle size), the eluent was detected at 232 nm with a run time of 10 min and a 20 µl injection volume [17].

Determination of Levocetirizine in Human Plasma by LC–MS-MS

Wichitnithad et al. [18]: a method using an isocratic mobile phase of acetonitrile and 10 mm ammonium formate pH 3.5 (80:20, v/v) at a flow rate of 1.0 ml/min, chromatographic separation was carried out on a reverse phase column. There was a 3.5-min runtime. Levocetirizine transitions at m/z (M1H)1 389.0!201.0 and hydroxyzine transitions at m/z (M1H) 1 375.3!201.0 were monitored using optimised mass parameters as an internal standard. The dynamic range and the lower bound of quantification were 1.00 and 1.00–500 ng/ml, respectively. For both intraday and inter-day validations, linearity was good (r^2=0.995). Levocetirizine and hydroxyzine had mean recoveries of 59 and 69%, respectively. With %CV<15, the matrix effect was deemed acceptable. The haemolytic effect was minimal. Levocetirizine was stable in human plasma for three freeze/thaw cycles: 16 weeks frozen at -270°C, 4 weeks frozen at -220°C, 27 h in an auto sampler at -15°C, and 27 h at room temperature (25°C). Levocetirizine concentrations in plasma samples were measured using the approved method in a pharmacokinetic investigation. The work offers a quick and easy bioanalytical technique for regular analysis, which could be very helpful for bioequivalency research [18].

Isocratic RP-HPLC Method

Thirunarayanan et al. [19]: by utilising a column, Phenomenex Luna-C 18 (250 mm×4.6 mm; 5 µ), and a mobile phase consisting of a buffer and acetonitrile mixture (580:420), the filtration procedures are assessed using the reverse phase isocratic HPLC method. The pH is adjusted to 6.0 with 10% sodium hydroxide. Buffer: 600 ml of water with 4.08 gm of potassium dihydrogen orthophosphate. The eluant is being monitored at 230 nm using a UV detector, and the flow rate is 1.0 ml/min. Levocetirizine HCl has a retention time of 5.5. According to precision, the relative standard deviation is 1.1%. Levocetirizine has been recovered 99.6% of the way from the dose formulation. Levocetirizine HCl's robustness and ruggedness test results fall comfortably within the acceptable range. It has been discovered that the suggested method is easy to use, quick, exact, and accurate. The suggested techniques are effectively used for determining the Levocetirizine tablet and have been validated in accordance with ICH recommendations.

Chromatographic conditions and measurement procedure:

- *Column:* Phenominex luna (250×4.6 mm), 5 µ.
- *Column oven temperature:* Ambient.
- *Flow rate:* 1.0 ml/min.
- *Wave length:* 230 nm.
- *Injection volume:* 20 µl [19].

Simultaneous Determination by Spectrophotometric Methods

Rashed et al. has developed the 1st approach using a bivariate calibration algorithm to determine the medications under study by using two chosen wavelengths: 220 and 230 nm [20]. The 2nd method, called dual wavelength, is based on measuring Levocetirizine dihydrochloride at two different absorbance differences: 208 and 214.4 nm, where the Montelukast sodium absorbance difference was zero for any concentration, and 355 and 390 nm, where the Levocetirizine dihydrochloride absorbance difference was zero for any concentration as well. Levocetirizine dihydrochloride and Montelukast sodium were measured using second derivative responses at 244 and 293.2 or 335.6 nm, respectively, in the 3rd approach, which is derivative spectrophotometry. Using 4 $\mu g.ml^{-1}$ Montelukast sodium as a divisor, the peak amplitudes for Levocetirizine dihydrochloride at 216 and 232 nm can be measured using the 4th method, ratio difference. Similarly, Montelukast sodium can be measured at 296.4 and 344.2 nm using 4 $\mu g.ml^{-1}$ of Levocetirizine dihydrochloride as a divisor. For both medications, Beer's law was followed in the concentration range of 4–28 $\mu g.ml^{-1}$ using all available methods. With good accuracy and precision, the proposed procedures were employed to identify the pharmaceuticals under study in bulk powder, laboratory-prepared mixes, and pharmaceutical dosage forms. The results were statistically compared to those obtained from a reference method and were found to be in good agreement. All methods were validated in accordance with ICH requirements [20].

First-Order Derivative Spectrophotometry for Simultaneous Determination

Parmar et al. [21]: the LCT and PHE were quantified using the first-order derivative absorption at 240 nm (the zero crossing point of PHE) and 283.2 nm (the zero crossing point of LCT), respectively. LCT and PHE were shown to be linear throughout concentration ranges of 4–24 and 8–48 g/ml, respectively, with correlation coefficients ($i2$) of 0.9964 and 0.992. The range of mean percentage recoveries for LCT and PHE was determined to be 99.14–100.43 and 98.73–100.83%, respectively. The suggested technique has been successfully used for the simultaneous estimation of LCT and PHE in combination tablets and has been validated in accordance with ICH guidelines [21].

Instruments: Using 1 cm-matched quartz cells, UV probe 2.34 software, and a Shimadzu UV-Visible 1700 PharmaSpec double beam spectrophotometer with a wavelength precision of ±0.3 nm, an analytical balance that is calibrated for weighing purposes, a Shimadzu BP211D (Sartorius Gottingen AG, Germany) was utilized. Microsoft Excel 2007 was used as the analytical tool for all statistical computations [21].

Liquid Chromatographic Method for Estimation

Chaitanya et al. [22]: The chromatographic system was made up of the Water 2695 binary gradient pump, the Water 2487 dual wavelength absorbance detector, and Empower 2 software. On the XTerra symmetry C18 column, separation was achieved at room temperature. Using a 20 µl sample loop and an injector valve, the sample was inserted. The outcomes demonstrated strong concurrence with the declared content. Levocetirizine recovery levels in tablet form ranged from 99.57 to 100%. The suggested technique is quick, precise, and specific; it can be applied to the quantitative examination of Levocetirizine in bulk medications, various dosage formulations, and raw materials [22].

Materials and instrumentation: Reddy's Laboratory in Hyderabad provided a complimentary sample of pure LTZ medication. We bought HPLC-grade acetonitrile from Merck in India. High-purity water was purified using Millipore technology. All additional analytical-grade chemicals and reagents used

in this experiment were sourced from Fine Chemicals, India. Throughout the analysis, the Water 2695 binary gradient pump, Water 2487 dual wavelength absorbance detector (DAD), built-in auto sampler, and column oven were all part of the chromatograph system. Empower-2 was the program used to collect the data. XTerra symmetry C18 (150×4.6 mm, 3.5 µm) was the column that was utilized. The mobile phase was composed of a 35:65% v/v combination of acetonitrile and phosphate buffer with a pH adjustment set at 3.0. A flow rate of 0.7 ml/min was selected. A 20 µl sample was injected onto the HPLC column, and the flow rate was adjusted to 0.7 ml/min. The retention period for LTZ was found to be 2.552 min when the eluent was measured at 230 nm [22].

HPLC and HPTLC Methods for Simultaneous Determination

Rathore, et al. [23]: the technique involved utilising orthophosphoric acid as the mobile phase and disodium hydrogen phosphate buffer (0.02 M): methanol (25:75, v/v) with pH adjusted to 7 for HPLC separation on a BDS Hypersil C18 column. The suggested techniques were successfully used for identifying the medications under investigation in tablets after being validated in accordance with ICH recommendations.

Instrumentation and chromatographic conditions: In order to perform HPTLC, the samples were spotted using a Camag Linomat IV applicator (Switzerland) in the shape of 6 mm wide bands on silica gel-precoated aluminium plate 60 F254 ((20×10 cm) with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Techno, Mumbai). Before chromatography, the plates were activated for 5 minutes at 110°C and prewashed with methanol. The gap between the two bands was 6 mm, and a constant application rate of 0.1 $l.s^{-1}$ was employed. Slit size was maintained at 5 mm×0.45 mm, and scanning speed was 10 $mm.s^{-1}$. For chromatography, 15 ml of the mobile phase, which included toluene, ethyl acetate, methanol, and ammonia (2.5: 7: 2.5: 1, v/v/v/v), were utilized. A glass chamber measuring 20 cm×10 cm and saturated with the mobile phase was used to conduct a linear ascending development (Camag, Muttenz, Switzerland). The optimal chamber saturation time for the mobile phase was found to be 30 min at room temperature (25±2°C).

The chromatogram run measured 8 cm in length. Densitometric scanning was carried out with a Camag TLC scanner III running CATS software (V 3.15, Camag) in the reflectance-absorbance mode. An uninterrupted UV spectrum spanning from 190 to 400 nm was produced by a deuterium lamp, which served as the radiation source. To compute the concentrations of the substances chromatographed, diffuse light intensity was used. Linear regression was used to evaluate the peak areas. A pump (Jasco PU-2080 Plus model) and a manual injector sampler set to a 20 µl injection capacity each comprised the HPLC system (Jasco Corporation, Tokyo, Japan). UV/VIS (model Jasco UV 2075) was the type of detector used. The BDS Hypersil C18 analytical column (Thermo Scientific, Waltham, USA) with dimensions of 250×4.6 mm and a particle size of 5 µ was used for the LC separations. Using the LC-Net II/ADC system and Jasco Borwin version 1.5, the data was merged. The sodium dihydrogen phosphate buffer (0.02 M) and methanol (25:75, v/v) were combined to create the mobile phase, which had its pH adjusted to 7 using orthophosphoric acid. Before usage, the mobile phase was filtered and degassed by passing through a Millipore membrane filter with a 0.45 m pore size (Milford, MA, USA). 1 $ml.min^{-1}$ was the flow rate. With a detection wavelength of 231 nm, all calculations were carried out at room temperature [23].

CONCLUSION

This review elucidates the significance of RP-HPLC in the estimation of Levocetirizine and related compounds, emphasizing the robustness of the analytical method. The UV detection employed in this study not only enhances specificity but also contributes to the overall precision and accuracy of the estimation process. The comprehensive exploration of method development and validation underscores the applicability and reliability of RP-HPLC in pharmaceutical analysis. As researchers continue to advance analytical techniques, this review serves as a valuable resource, consolidating key insights for future investigations in the field of simultaneous estimation using RP-HPLC.

REFERENCES

1. Pathuri R, Muthukumaran M, Krishnamoorthy B, Nishat A. A review on analytical method development and validation of pharmaceutical technology. J Curr Pharma Res. 2013; 3(2): 855.
2. Shivhare Himani, Preeti Chincholikar. Analytical chemistry: Overview (Techniques & Applications). J Pharm Negat Results. 2022; 13(4): 1364–1373.
3. Panchal DMK, Patel D, Patel K, Dalwadi M, Upadhyay U. A review on "high performance thin layer chromatography". World J Pharm Res. 2022; 11(15): 282–297.
4. Vidushi Y, Meenakshi B, Bharkatiya M. A review on HPLC method development and validation. Res J Life Sci, Bioinform, Pharm Chem Sci. 2017; 2(6): 166–178.
5. Rina R, Baile M, Jain AA. Review: Analytical Method Development and Validation. Syst Rev Pharm. 2021 Jun 11; 12(11): 3601–5.
6. Shetgar SS, Ramadevi D, Rao BM, Basavaiah K. RP-UPLC method development and validation for quantitative estimation of levocetirizine in levocetirizine dihydrochloride tablets. Int J Pharm Sci Res (IJPSR). 2022; 13(2): 987–95.
7. Suyash Gupta, *et al.* Method Development and Validation of an RP- HPLC Method for Determination of Levocetirizine. Int J Pharm Pharm Res (IJPPR). 2022; 26(1): 147–182.
8. Prasada Rao M. Analytical Method Development and Validation of Levocetirizine Hydrochloride and Montelukast Sodium in Combined Tablet Dosage Form by RP-HPLC. Res J Pharm Biol Chem Sci. 2014; 5(6): 1010–1021.
9. Shetgar SS, Ramadevi D, Rao BM, Basavaiah K. RP-UPLC method development and validation for quantitative estimation of levocetirizine in levocetirizine dihydrochloride tablets. Int J Pharm Sci Res (IJPSR). 2022; 13(2): 987–95.
10. Biagi M, Vegni AM, Pergoloni S, Butala PM, Little TD. Trace-orthogonal PPM-space time block coding under rate constraints for visible light communication. J Lightwave Technol. 2014 Dec 31; 33(2): 481–94.
11. Kumar S, Gautam D, Talwan P. Method development and validation for the simultaneous estimation of in atorvastatin and fenofibrate bulk and pharmaceutical dosage form by using RP-HPLC method. Int J Res Pharm Chem. 2020; 10(1): 28–34.
12. Ali S, Gupta M. Method development and validation for the simultaneous estimation of montelukast sodium and levocetirizine hydrochloride tablet using RP-HPLC. J Pharm Sci Res. 2019 Aug 1; 11(8): 2998–3000.
13. Venila Rani K, *et al.* Method development and validation for the simultaneous estimation montelukast and levocetirizine pharmaceutical dosage form by using RP-HPLC. Int J Pharm. 2015; 5(2): 631–636.
14. Basu A, Basak K, Chakraborty M, Rawat IS. Simultaneous RP-HPLC estimation of Levocetirizine Hydrochloride and Montelukast Sodium in tablet dosage form. Int J PharmTech Res. 2011 Jan; 3(1): 405–10.
15. Sonawane JK, Patil DA, Jadhav BS, Jadhav SL, Patil PB. Stability Indicating RP-HPLC method development and validation for simultaneous quantification of antihistaminic & anti-asthmatic drug in bulk and tablet dosage form. J Pharm Biol Sci. 2020; 8(1): 12–22.
16. Madasu SD, Sai Kumar MLS, Singh AK. A flower pollination algorithm based automatic generation control of interconnected power system. Ain Shams Eng J. 2018;9:1215–1224. doi: 10.1016/j.asej.2016.06.003.
17. Khatri S, Dhanoriya C, Jain DK. Zika virus (ZIKV) disease: past, present and future. J Drug Deliv Ther. 2018 Dec 15; 8(6–s): 320–7.
18. Wichitnithad W, Jithavech P, Sanphanya K, Vicheantawatchai P, Rojsitthisak P. Determination of Levocetirizine in Human Plasma by LC–MS-MS: Validation and Application in a Pharmacokinetic Study. J Chromatogr Sci. 2015 Nov 1; 53(10): 1663–72.
19. Rajarajana M, Senbagam R, Vijayakumar R, Manikandan V, Balaji S, Vanangamudi G, Thirunarayanan G. Synthesis, spectral studies and antimicrobial activities of some substituted (E)-1-benzylidene-2-(4-bromophenyl) hydrazines. World Sci News. 2015; (3): 155–71.
20. Rashed Noha S, Abdallah Ola M, Said Noha S. Analytical Chemistry Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4(05).

21. Akter F, Mannan A, Lipi N, Rahman NA, Lugova H, Haq MA, Haque M. Proficiency and implementation associated with non-communicable diseases among secondary school students in Bangladesh. J Appl Pharm Sci. 2023;13:162–175.
22. Chaitanya Prasad MK, Vidyasagar G, Sambasiva Rao KRS, Madhusudhana Reddy Induri and Ramanjeneyulu S. Development of validated liquid chromatographic method for estimation of levocetirizine from pharmaceutical dosage forms. J Appl Pharm Sci. 2011; 01(10): 95–97.
23. Rathore AS, Sathiyanarayanan L, Mahadik KR. Development of validated HPLC and HPTLC methods for simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in bulk drug and pharmaceutical dosage form. Pharm Anal Acta. 2010; 1(1): 1–6.

