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Review Article

A Comprehensive Review on RP-UPLC Method Development and Validation of Bilastine

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Bilastine is a second-generation, selective peripheral histamine H1-receptor antagonist used for the symptomatic treatment of allergic rhino-conjunctivitis and urticaria. Reliable analytical procedures are essential for confirming the quality and consistency of pharmaceutical products containing Bilastine. RP-UPLC combines reversed-phase chromatographic selectivity with the high efficiency and reduced analysis time achievable using small-particle columns and suitable high-pressure instrumentation. This review discusses the physicochemical and analytical considerations relevant to Bilastine, the principles and instrumentation of RP-UPLC, systematic method development, chromatographic optimization, and analytical procedure validation. Particular attention is given to stationary-phase selection, mobile-phase composition, pH, organic modifier, flow rate, detection wavelength, injection volume, column temperature and run time. Validation characteristics including specificity, system suitability, linearity and range, accuracy, repeatability, intermediate precision, robustness, limit of detection, limit of quantification and solution stability are reviewed in the context of ICH Q2(R2) and ICH Q14. The review also considers the advantages and limitations of RP-UPLC compared with conventional HPLC and highlights its potential value for rapid routine pharmaceutical quality-control analysis.

Keywords: Bilastine; RP-UPLC; analytical method development; method validation; pharmaceutical analysis; linearity; accuracy; precision; robustness; LOD; LOQ.

INTRODUCTION

Pharmaceutical analysis is concerned with the identification, characterization, quantification and quality assessment of drug substances, excipients, impurities and finished dosage forms. An analytical procedure used in pharmaceutical quality control must be scientifically sound and appropriate for its intended purpose. The analytical result should be sufficiently reliable to support decisions concerning identity, strength, purity, release and, where applicable, stability. Chromatographic procedures are particularly important because they can separate a target analyte from structurally related compounds, degradation products and formulation components. High-performance liquid chromatography (HPLC) has become one of the principal analytical techniques in pharmaceutical laboratories because it accommodates compounds with a wide range of polarity, molecular size and thermal stability. Reversed-phase HPLC is especially versatile because non-polar stationary phases can be combined with aqueous-organic mobile phases, allowing retention to be controlled by solvent composition, pH and other variables [1-5]. The development of ultra-performance liquid chromatography (UPLC) represented an important advancement in liquid chromatographic technology. Smaller stationary-phase particles can provide higher efficiency and allow useful separations to be achieved with shorter columns, higher linear velocities and reduced solvent consumption. Swartz described UPLC as an approach capable of improving speed, resolution and sensitivity while maintaining the fundamental separation mechanisms of liquid chromatography [6]. For pharmaceutical applications, the benefit of UPLC is

not simply a shorter run time. A well-designed method should provide adequate retention and selectivity, symmetrical peaks, reproducible response, acceptable system pressure and sufficient separation from potential interferences. Consequently, UPLC method development is best regarded as a controlled optimization process rather than a simple substitution of an HPLC column with a smaller particle-size column.

1.1 Need for an Analytical Method for Bilastine

Bilastine is a modern second-generation antihistamine. Its analysis in pharmaceutical dosage forms requires consideration of the drug's chemical structure, ionization behavior, chromatographic retention and ultraviolet response. Formulations may contain diluents, binders, lubricants, coating materials or other excipients that can affect extraction and chromatographic behavior. A suitable RP-UPLC procedure should therefore be able to distinguish the Bilastine response from blank and placebo components and provide reproducible quantification. The present review provides the theoretical foundation for an M. Pharm project focused on RP-UPLC method development and validation of Bilastine. The emphasis is on a prospective method-development framework; project-specific chromatographic values should be reported separately after experimental optimization.

1.2 Objectives of the Review

- To describe the analytical and pharmaceutical importance of Bilastine.
- To explain the principles and instrumentation of RP-UPLC.
- To discuss the major variables used during method development and optimization.
- To summarize current expectations for validation of quantitative analytical procedures.
- To provide a scientific framework for the proposed RP-UPLC determination of Bilastine in pharmaceutical formulations.

2. Bilastine: Pharmaceutical and Chemical Profile

Bilastine is a second-generation H1-antihistamine developed for the treatment of allergic conditions. It acts predominantly at peripheral H1 receptors and is associated with the pharmacological profile expected of a modern, relatively non-sedating antihistamine. Its therapeutic use includes symptomatic relief in allergic rhino-conjunctivitis and urticaria. The pharmacological properties of Bilastine are relevant to analytical science because the molecule contains multiple functional groups that influence polarity, hydrogen bonding and ionization, all of which may affect reversed-phase chromatographic behavior. The molecular formula of Bilastine is C28H37N5O3 and its molecular mass is approximately 463.62 g/mol. Structural information is useful during analytical method development because it helps guide initial decisions about stationary phase, aqueous-organic mobile phases and pH. However, chromatographic conditions should ultimately be selected on the basis of experimental selectivity, peak shape and reproducibility rather than structure alone.

2.1 Analytical Significance of Physicochemical Properties

In reversed-phase chromatography, hydrophobic interactions with the stationary phase generally increase retention, whereas increasing the proportion of organic solvent commonly decreases retention. Ionization can substantially modify apparent retention because charged species usually interact differently with a non-polar stationary phase than their neutral forms. Consequently, pH selection may be an important development variable for an ionizable pharmaceutical compound. For a pre-project review, it is preferable to describe these relationships conceptually rather than assign an experimental retention time to Bilastine before the method has been developed. Actual retention time, theoretical plate count, tailing factor and detector response should be obtained experimentally under the final optimized conditions.

2.2 Pharmaceutical Dosage Forms

Bilastine is commercially available primarily as an oral pharmaceutical product. The dosage-form matrix can influence sample preparation and chromatographic performance. Excipients may contribute to background response, adsorption, extraction variability or unexpected peaks. A validated procedure should therefore demonstrate that

the sample matrix does not produce significant interference at the analyte's chromatographic position.

2.3 Analytical Method Requirements

Requirement	Purpose
Specificity	Demonstrate that Bilastine can be measured in the presence of relevant matrix components.
Selectivity	Provide adequate chromatographic discrimination from potential related substances or formulation peaks.
Sensitivity	Support detection and quantification at the intended concentration levels.
Precision	Demonstrate reproducibility of the measurement procedure.
Accuracy	Demonstrate closeness of measured results to the accepted reference value.
Robustness	Assess reliability under small deliberate changes in method conditions.

These requirements provide the conceptual bridge between pharmaceutical characteristics and the chromatographic method-development strategy.

Principles Of RP-UPLC

Reversed-phase ultra-performance liquid chromatography combines the retention mechanism of reversed-phase liquid chromatography with instrumentation and columns designed for efficient operation at high pressure. In a typical reversed-phase system, the stationary phase is relatively non-polar and the mobile phase is comparatively polar. Common stationary phases include alkyl-bonded silica materials such as C18. Analytes partition or interact between the mobile and stationary phases to different extents, resulting in chromatographic separation. The high efficiency of UPLC is strongly associated with the use of small stationary-phase particles. Smaller particles reduce the distance available for mass transfer and can increase column efficiency. At the same time, they increase resistance to flow and therefore require instrumentation capable of operating at higher pressures. The practical result is a system that can achieve high efficiency with short columns and relatively rapid analyses [6,7].

3.1 Major Components of an RP-UPLC System

Solvent reservoirs: contain the mobile-phase components and must be compatible with the chromatographic system.

Degassing system: minimizes dissolved-gas-related problems such as bubble formation and detector instability.

Pump: delivers the mobile phase at a controlled flow rate and, where required, mixes solvent components accurately.

Autosampler or injector: introduces a reproducible volume of sample into the flowing mobile phase.

Analytical column: contains the stationary phase and provides the separation.

Column oven: maintains a controlled temperature when temperature control is part of the method.

Detector: converts the eluting analyte into a measurable signal; UV and PDA detectors are commonly used in pharmaceutical liquid chromatography.

Data system: records chromatographic signals and calculates retention time, area and system-suitability parameters.

3.2 Basic Chromatographic Parameters

Parameter	Analytical significance
Retention time	Helps identify the chromatographic position of the analyte under defined conditions.
Peak area	Usually related to analyte amount in quantitative chromatographic analysis.
Theoretical plates	Indicator of chromatographic efficiency.
Tailing factor	Indicator of peak symmetry.

Resolution	Measures separation between adjacent chromatographic peaks.
Capacity/retention factor	Describes analyte retention relative to the mobile phase.

A successful RP-UPLC method should balance these parameters. Very short retention may increase the risk of interference, whereas excessive retention can reduce throughput. High efficiency is useful only if it is accompanied by adequate selectivity and reproducible peak shape.

Detectors Used In UPLC

The detector is responsible for converting the physical presence of an eluting analyte into an electrical signal that can be recorded and processed. Detector selection depends on the analyte's chemical properties, the required sensitivity, selectivity, concentration range and intended application. UV/visible detection remains particularly common in pharmaceutical laboratories because many drug molecules possess chromophores and because UV detectors are comparatively simple to operate.

4.1 UV Detector

A variable-wavelength UV detector monitors absorbance at a selected wavelength. It is useful when the analyte has a suitable absorption maximum and when the formulation matrix does not create substantial interference at that wavelength. During method development, the wavelength should be selected to provide adequate analyte response while minimizing background response.

4.2 Photodiode Array Detector

A photodiode array (PDA) detector can acquire absorbance information over a range of wavelengths. This provides additional spectral information compared with single-wavelength detection. PDA data can be useful for evaluating peak purity and for confirming that the selected wavelength is appropriate. It can also assist method development by enabling the analyst to inspect the UV spectrum of the analyte and matrix.

4.3 Other Detector Classes

Other detectors used in liquid chromatography include fluorescence, refractive-index, evaporative light-scattering, charged-aerosol and mass-spectrometric detectors. Their use depends on the analyte and the purpose of the procedure. Mass spectrometry can provide high selectivity and structural information but generally requires greater instrument complexity and method-development expertise.

Detector	Typical strength	General limitation
UV/Vis	Simple, robust and widely applicable to chromophoric drugs	Requires adequate UV absorption and may have matrix interference
PDA	Multi-wavelength acquisition and spectral information	More data handling; still dependent on optical absorption
Fluorescence	High sensitivity for fluorescent analytes	Limited to suitable fluorophores or derivatized analytes
RI	Useful for compounds with weak UV absorption	Less sensitive and more temperature dependent
MS	High selectivity and structural information	Higher cost and operational complexity

5. RP-UPLC Method Development: Strategy and Optimization

Analytical method development should proceed from a clear analytical target profile. The intended measurement, dosage form, concentration range, expected matrix, required sensitivity and reporting purpose should be defined before optimization begins. ICH Q14 describes a science- and risk-based framework for analytical procedure development and encourages understanding of the relationship between method parameters and analytical performance [15].

5.1 Selection of Stationary Phase

C18 reversed-phase columns are commonly considered for pharmaceutical compounds because of their broad applicability and reproducibility. However, not all C18 columns behave identically. Differences in silica surface properties, bonding density, end-capping, pore structure and particle technology can influence retention and peak shape. Column selection should therefore be based on observed selectivity rather than brand name alone.

5.2 Mobile-Phase Development

The mobile phase may contain an aqueous buffer and an organic modifier such as acetonitrile or methanol. Organic composition is a major control variable because it changes analyte retention. A systematic screening approach can compare different organic modifiers and different aqueous-to-organic ratios. The final choice should provide suitable retention, peak symmetry, resolution and run time.

5.3 Buffer and pH

For ionizable analytes, pH can strongly affect retention and peak shape. The buffer should have adequate capacity near the selected pH and should be compatible with the detector and column. When using UPLC, the buffer concentration should also be selected with attention to system pressure, column compatibility and laboratory practice.

5.4 Flow Rate

Flow rate influences retention time, efficiency and back pressure. The optimum should be established experimentally within the operating limits of the column and instrument. Increasing flow rate may reduce analysis time but can also change efficiency and pressure. A development study should therefore assess the overall chromatographic response rather than optimizing speed alone.

5.5 Column Temperature

Temperature can alter solvent viscosity, mass transfer and analyte-stationary-phase interactions. Controlled temperature may improve reproducibility and can sometimes improve peak shape. If temperature is used as a method parameter, it should be defined clearly and controlled throughout validation.

5.6 Injection Volume and Run Time

Injection volume should be sufficiently large to obtain an appropriate detector response but not so large that it causes overload or peak distortion. The run time should allow the analyte and relevant potential interferences to elute while avoiding unnecessary solvent use.

6. Systematic Optimization of The Bilastine Method

Optimization should be performed through sequential or design-based experiments rather than by changing several variables without recording their effects. A practical laboratory approach begins with scouting experiments and then narrows the conditions to a small region that provides acceptable chromatographic behavior. If resources permit, a design of experiments (DoE) approach can be used to study interactions between factors and identify a robust operating region.

6.1 Suggested Development Sequence

1. Confirm the identity and approximate concentration of the intended Bilastine solution.
2. Review the UV absorption characteristics and select a preliminary detection region.
3. Screen a suitable reversed-phase column, commonly beginning with a C18 chemistry.
4. Evaluate aqueous-organic mobile phases using compatible buffers and organic modifiers.
5. Investigate pH where ionization may materially affect retention and peak shape.
6. Adjust flow rate and temperature to obtain an efficient and practical separation.
7. Optimize injection volume and total run time.
8. Compare blank, placebo and sample chromatograms to evaluate potential interference.
9. Select provisional conditions and challenge them with small deliberate variations.

10. Document the final conditions as the proposed analytical procedure for validation.

6.2 Chromatographic Response Criteria

The principal responses during optimization are retention time, peak area response, peak symmetry, theoretical plate count and resolution. The relative importance of each response depends on the analytical purpose. For a simple assay method, adequate specificity, precision and accuracy may be more important than achieving maximum resolution between peaks that are not relevant to the intended measurement. For a related-substances method, resolution and selectivity become much more critical.

Development variable	Possible effect on chromatogram
Organic solvent proportion	Changes retention and often selectivity
Organic solvent identity	Can change selectivity, peak shape and pressure
pH	Changes ionization and therefore retention/shape for ionizable compounds
Flow rate	Changes retention, efficiency and pressure
Column chemistry	Changes selectivity and retention
Temperature	Can change retention, viscosity and efficiency
Injection volume	Can affect response and peak distortion
Run time	Determines completeness of elution and throughput

6.3 Avoiding Over-Optimization

A method should not be optimized solely for one chromatogram. A condition that gives an attractive peak under one exact setting may be fragile when the flow rate, wavelength, temperature or mobile-phase composition changes slightly. Robustness should therefore influence the final choice of conditions. The preferred method is usually the one that provides an acceptable performance region rather than a single narrow optimum.

7. Sample Preparation and Specificity

Sample preparation is an integral part of an analytical procedure. Even a highly efficient chromatographic method can give unreliable results if the analyte is extracted inconsistently or if the sample solution contains substances that interfere with measurement. For Bilastine dosage forms, the sample preparation should be designed to obtain representative extraction while minimizing unnecessary manipulation.

7.1 General Sample-Preparation Considerations

- Use a representative quantity of dosage-form material.
- Employ a diluent compatible with the mobile phase and analyte solubility.
- Use controlled mixing, sonication or shaking when required for complete extraction.
- Allow sufficient time for insoluble excipients to settle or use a suitable filtration step when justified.
- Evaluate filter compatibility so that adsorption or extractables do not alter the result.
- Prepare standard and sample solutions in a manner that minimizes concentration errors.

7.2 Specificity

Specificity is the ability of the analytical procedure to measure the analyte unequivocally in the presence of components that may be expected to be present. For a Bilastine assay, specificity is commonly considered by comparing chromatograms of diluent or blank, placebo, standard and sample preparations. The critical observation is whether any blank or placebo response occurs at the chromatographic position used for Bilastine quantification. Where a PDA detector is available, spectral information can provide additional evidence that the analyte peak is spectrally consistent. For stability-indicating applications, specificity should also consider relevant degradation products and forced-degradation samples where appropriate.

7.3 Placebo and Blank Studies

Preparation	Purpose
Diluent/blank	Check for response arising from solvent and mobile-phase components.
Placebo	Assess interference from formulation excipients.

Standard	Establish analyte response and chromatographic position.
Sample	Demonstrate performance in the actual dosage-form matrix.
Spiked sample	Support assessment of recovery and matrix effects.

7.4 Filter Compatibility

Filtration can introduce bias through analyte adsorption or extractable materials. A filter-compatibility assessment should compare filtered and appropriately centrifuged or otherwise prepared solutions, where scientifically justified. The selected filter should not significantly change the measured Bilastine response.

7.5 Solution Stability

Standard and sample solutions may be evaluated for stability over the intended period and under defined storage conditions. The purpose is to establish whether the analytical solution remains suitable during the normal sequence of preparation and analysis. Stability conclusions should be based on experimental comparison with freshly prepared reference solutions.

8. Method Validation: General Principles

Method validation provides documented evidence that an analytical procedure is suitable for its intended purpose. ICH Q2(R2) describes validation characteristics and emphasizes that the extent of validation should reflect the intended use of the analytical procedure [14]. ICH Q14 complements Q2(R2) by describing a scientific approach to analytical procedure development and lifecycle management [15].

8.1 Validation Characteristics Relevant to a Bilastine Assay

Parameter	What it demonstrates
Specificity	Ability to measure Bilastine without significant interference from relevant components.
System suitability	Adequate performance of the chromatographic system before or during analysis.
Linearity / response	Relationship between analyte concentration and analytical response over the intended range.
Range	Concentration interval over which the method demonstrates suitable performance.
Accuracy	Closeness of the result to the accepted reference value.
Repeatability	Precision under the same operating conditions over a short interval.
Intermediate precision	Precision under within-laboratory variations such as day, analyst or equipment.
Robustness	Ability to remain reliable after small deliberate changes in method parameters.
LOD	Lowest amount that can be detected under the defined procedure.
LOQ	Lowest amount that can be quantified with suitable performance.

8.2 System Suitability

System suitability testing verifies that the chromatographic system is functioning adequately for the intended analysis. Commonly monitored characteristics include retention time, peak area, theoretical plates, tailing factor and %RSD for replicate standard injections. The actual acceptance limits should be predefined in the analytical procedure and justified according to the method purpose and applicable pharmacopoeial or regulatory expectations. For an M. Pharm project, six replicate injections of the standard solution may be used to demonstrate repeatability of chromatographic response when consistent with the selected protocol. The resulting mean, standard deviation and %RSD should be reported. Project-specific numerical values should appear in the experimental results section, not in the pre-project literature review.

8.3 Specificity and System Suitability Relationship

Specificity examines the measurement in the presence of potential interferents, whereas system suitability examines whether the chromatographic system is performing adequately. Both are important but they answer different questions. A system can have

excellent repeatability while still failing specificity if a placebo peak overlaps the analyte response.

9. Linearity, Range and Calibration

Linearity describes the ability of an analytical procedure to obtain responses that are proportional to the amount of analyte within a specified concentration interval. For a quantitative Bilastine procedure, standard solutions covering the intended analytical range can be prepared and analyzed under the same chromatographic conditions. Peak response is then related to concentration using an appropriate regression model.

9.1 Calibration Curve

A calibration plot commonly presents Bilastine concentration on the x-axis and chromatographic response, such as peak area, on the y-axis. The regression equation can be expressed as $y = mx + c$, where y is the response, x is concentration, m is slope and c is intercept. The slope reflects analytical sensitivity over the studied interval, while the intercept provides information about systematic response at zero concentration. The correlation coefficient or coefficient of determination is useful as a descriptive statistic, but it should not be interpreted as the only evidence of linearity. Residuals, response distribution, back-calculated concentrations and the suitability of the regression model should also be considered. ICH Q2(R2) emphasizes demonstrating the performance characteristics relevant to the intended analytical procedure rather than relying on a single statistical number [14].

9.2 Proposed Linearity Levels

The exact levels should be aligned with the intended assay range and project protocol. A common academic design may examine several levels distributed across the working range, for example 50%, 80%, 100%, 120% and 150%, when scientifically justified. The final levels should be defined before the study and should be consistent with the concentration used for the proposed assay.

Level	Purpose
Low level	Assesses response near the lower end of the intended range.
Intermediate level	Provides information around the target assay concentration.
Target level	Represents the nominal working concentration.
Upper levels	Evaluate proportional response toward the upper end of the intended range.

9.3 Range

Range is the interval between the lowest and highest concentrations for which the analytical procedure demonstrates suitable performance for the intended purpose. For an assay method, the range should encompass the expected sample concentration and any relevant variation introduced by sample preparation or analytical use.

9.4 Interpretation

A sui table linearity study should show an appropriate relationship between concentration and response, without meaningful systematic curvature across the intended range. The final project report should include the individual observations, regression equation, correlation statistic and graphical calibration plot.

10. Accuracy and Precision

10.1 Accuracy

Accuracy expresses the closeness of agreement between the result obtained by the analytical procedure and the accepted reference value. In pharmaceutical assay validation, accuracy is frequently assessed through recovery studies in which known quantities of Bilastine standard are added to a pre-analyzed sample or placebo matrix. Multiple concentration levels and replicate preparations are generally used. A typical academic design may include three concentration levels, such as 50%, 100% and 150% of the target concentration, with triplicate preparations at each level, provided these levels are appropriate for the intended procedure. The percentage recovery is calculated for each preparation, followed by evaluation of the mean

recovery and variability. The acceptance limits should be established prospectively based on the method purpose and applicable guidance.

10.2 Precision

Precision describes the closeness of agreement among a series of measurements obtained from multiple sampling of the same homogeneous material under specified conditions. Precision is commonly expressed as standard deviation, variance or %RSD.

10.2.1 Method Precision (Repeatability)

Method precision evaluates repeatability under the same operating conditions. For an M.Pharm assay project, multiple independent sample preparations may be analyzed on the same instrument and under the same method conditions. The assay results and, where appropriate, chromatographic responses are summarized using mean, standard deviation and %RSD.

10.2.2 Intermediate Precision

Intermediate precision evaluates variation within the same laboratory when relevant factors are changed. Examples include different days, analysts, instruments or columns. The study should be designed so that the selected sources of variation are clearly documented. The purpose is not to create arbitrary variability but to demonstrate that normal laboratory changes do not compromise the reliability of the method.

Precision study	Typical controlled factor
Repeatability	Same analyst, equipment, day and method conditions.
Intermediate precision	Different day and/or analyst and/or equipment within the same laboratory.
Reproducibility	Between laboratories; generally relevant to collaborative or standardization studies.

10.3 Reporting

A professional report should provide individual results, mean, standard deviation and %RSD. Where assay is the final reported result, the assay values should be shown for each preparation. If chromatographic parameters are also important, retention time, theoretical plates and tailing factor may be presented separately as system-performance data.

11. Robustness, LOD and LOQ

11.1 Robustness

Robustness is the ability of an analytical procedure to remain unaffected by small deliberate variations in method parameters. It is an important aspect of method development because a method that works only at one exact combination of conditions may be difficult to transfer or reproduce. ICH Q2(R2) links robustness to the reliability of the analytical procedure under normal variations [14]. For an RP-UPLC method for Bilastine, potential variables may include flow rate, detection wavelength, organic-phase proportion, pH and column temperature. Only scientifically relevant variables need to be investigated. The direction and magnitude of each change should be predefined.

Variable	Example of deliberate change	Potential response
Flow rate	Small increase/decrease around target	Retention, efficiency, peak shape
Detection wavelength	Small wavelength shift	Detector response and specificity
Organic phase	Small composition change	Retention and selectivity
pH	Small controlled change	Retention and peak shape
Temperature	Small controlled change	Retention and efficiency

Robustness assessment should focus on whether the method remains fit for purpose. The decision should consider assay or response, retention behavior, peak shape and other relevant system-suitability characteristics.

11.2 Limit of Detection

LOD is the lowest amount of analyte in a sample that can be detected, although it may not be quantitatively determined with suitable precision and accuracy. LOD is especially relevant when the procedure is intended to detect low-level impurities or when sensitivity is an explicit method requirement. ICH Q2(R2) provides approaches for estimating detection capability, including signal-to-noise concepts where applicable and calculation-based approaches using the standard deviation of the response and slope. The chosen approach should be appropriate to the analytical procedure and documented.

11.3 Limit of Quantification

LOQ is the lowest amount of analyte that can be quantitatively determined with suitable accuracy and precision. The LOQ should be confirmed experimentally, particularly when it is important to demonstrate quantitative performance at the proposed limit. Precision and accuracy at or near the LOQ provide useful evidence that the calculated value is practically meaningful.

12. Comparison of HPLC and UPLC

UPLC evolved from the same fundamental chromatographic principles used in HPLC but applies high-efficiency small-particle columns and instrumentation designed for higher operating pressures. The practical consequences include potentially faster separations, higher efficiency and lower solvent use. These advantages can be valuable in pharmaceutical laboratories where large numbers of samples are analyzed.

Feature	Conventional HPLC	UPLC
Particle size	Typically, larger particles	Small particles, commonly sub-2 µm in high-efficiency formats
Operating pressure	Lower than UPLC systems	Higher; requires suitable instrumentation
Analysis time	Often longer	Potentially shorter
Efficiency	High	Very high when appropriately optimized
Solvent consumption	Higher for comparable long methods	Often reduced because of shorter columns and faster runs
Sensitivity	Good; detector dependent	Can improve through efficient peak focusing and reduced dispersion
Method transfer	Established and widespread	Requires attention to column dimensions, flow, pressure and dwell volume

12.1 Advantages of RP-UPLC

- High chromatographic efficiency and sharp peaks.
- Shorter analysis time when method conditions are appropriately optimized.
- Reduced mobile-phase consumption in many applications.
- Potentially improved resolution and sensitivity.
- Good suitability for automated routine pharmaceutical analysis.
- Compatibility with UV/PDA and other advanced detectors.

12.2 LIMITATIONS

- Higher system pressure requires dedicated instrumentation and compatible columns.
- Small-particle columns can be more sensitive to inappropriate sample preparation or particulates.
- Method transfer between HPLC and UPLC requires careful adjustment rather than direct copying of conditions.
- Instrument and column costs may be higher.
- Buffers and sample matrices should be managed carefully to protect high-efficiency columns and system components.

13. Pharmaceutical Applications, Research Gap and Future Perspectives

13.1 Pharmaceutical Applications of RP-UPLC

RP-UPLC can be applied to a broad range of pharmaceutical analytical tasks. Depending on method design, applications may include assay of active pharmaceutical ingredients, identification or

monitoring of related substances, stability studies, content uniformity investigations and quality-control testing. The same platform can also support method-development experiments because it provides rapid feedback when chromatographic variables are changed. For Bilastine, an RP-UPLC assay procedure may be useful for routine testing of bulk drug or finished dosage forms. A sufficiently selective procedure could also provide a foundation for further work on stability-indicating analysis, provided specificity against relevant degradation products is experimentally established.

13.2 Research Gap

Although conventional chromatographic approaches are widely used in pharmaceutical analysis, an M.Pharm project can provide value by developing a procedure that is specifically optimized for the selected Bilastine dosage form, laboratory instrumentation and analytical objective. The research gap should not be stated as 'no method exists' unless a systematic literature search supports that claim. A more defensible rationale is that a project-specific RP-UPLC method can be optimized and validated for rapid, reproducible and economical routine analysis under the laboratory's intended conditions.

13.3 Future Perspectives

- Use of risk-based analytical procedure development in accordance with ICH Q14.
- Application of design of experiments to understand interactions among chromatographic parameters.
- Greater use of PDA spectral information for peak characterization.
- Integration of automated sample preparation and sequence monitoring.
- Development of stability-indicating methods when supported by degradation studies.
- Method lifecycle management, including continued performance monitoring after validation.

13.4 Proposed Validation Framework

Stage	Planned evaluation
Development	Column, mobile phase, pH, flow, wavelength, temperature and run time.
Specificity	Blank, placebo, standard and sample comparison.
System suitability	Replicate standard injections; retention, response, efficiency, symmetry and %RSD.
Linearity and range	Multiple concentration levels across intended working range.
Accuracy	Recovery at selected concentration levels.
Method precision	Independent replicate sample preparations.
Intermediate precision	Controlled within-laboratory variations.
Robustness	Small deliberate changes in critical method parameters.
LOD/LOQ	Sensitivity assessment where required by intended use.
Solution stability	Comparison of stored and freshly prepared solutions.

CONCLUSION

RP-UPLC is a powerful and efficient platform for pharmaceutical analysis because it combines reversed-phase separation principles with high-efficiency small-particle columns and high-pressure instrumentation. For Bilastine, a systematic method-development strategy should begin with a clear analytical objective and should consider the molecular and formulation characteristics that may influence chromatographic behavior. The selection of stationary phase, mobile phase, buffer, pH, organic modifier, flow rate, detection wavelength, temperature, injection volume and run time should be based on experimental evidence. A successful method should provide adequate retention, peak shape, efficiency, response and specificity while remaining practical for routine use. Importantly, method development should consider robustness so that the final procedure performs reliably within a reasonable operating region. Validation provides documented evidence that the developed procedure is fit for its intended purpose. Specificity, system suitability, linearity and range, accuracy, repeatability,

intermediate precision, robustness and, where applicable, LOD and LOQ should be evaluated in accordance with the analytical objective and relevant ICH guidance. Project-specific experimental results should be reported only after completion of the corresponding studies. Overall, the literature and established chromatographic principles support the use of RP-UPLC as a suitable platform for developing a rapid and reproducible analytical procedure for Bilastine. The proposed M.Pharm work can therefore focus on systematic optimization followed by validation and application to the selected pharmaceutical formulation.

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