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DEVELOPMENT AND VALIDATION OF API BY HPLC METHODS FOR THE QUALITY CONTROL TEST

Shruti Vilas Gawai[1] , Prof. Anjali Rathod[2]

[1]Department of Quality Assurance, Gawande College of Pharmacy, Sakharkherda, District- Buldhana, Maharashtra-443202, India.

[2]Professor Department of Quality Assurance, Gawande College of Pharmacy, Sakharkherda, District- Buldhana-443202, India.

Abstract:

High-Performance Liquid Chromatography (HPLC) is a widely applied analytical technique for the development and validation of methods used in the quality control of Active Pharmaceutical Ingredients (APIs). Reliable analytical procedures are essential to ensure the identity, purity, potency, and stability of APIs throughout manufacturing and storage. This review discusses systematic approaches to HPLC method development, including selection of column, mobile phase optimization, detection wavelength determination, and optimization of chromatographic parameters. It further highlights essential validation characteristics such as specificity, linearity, accuracy, precision, robustness, and system suitability in accordance with regulatory guidelines. The application of HPLC in assay determination, impurity profiling, stability studies, and dissolution testing is also summarized. Recent advancements including UPLC, LC–MS integration, green analytical approaches, and automation are addressed to emphasize modern trends in pharmaceutical analysis. Overall, validated HPLC methods play a crucial role in ensuring regulatory compliance, product consistency, and patient safety in pharmaceutical quality control.

Keywords: *Active Pharmaceutical Ingredient (API), High-Performance Liquid Chromatography (HPLC), Method Development, Method Validation, Quality Control, Impurity Profiling, Stability Studies, Regulatory Compliance*

Corresponding author:

Shruti Vilas Gawai,

Department of Quality Assurance, Gawande College of Pharmacy, Sakharkherda, District- Buldhana, Maharashtra-443202, India.

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1. INTRODUCTION:

The quality control of Active Pharmaceutical Ingredients (APIs) is a fundamental requirement in pharmaceutical manufacturing to ensure safety, efficacy, and batch-to-batch consistency of drug products. Regulatory authorities mandate that APIs meet predefined specifications for identity, purity, potency, and stability before they are used in formulation or released to the market. Analytical method development therefore plays a central role in pharmaceutical quality systems, as reliable and validated analytical procedures are essential for routine testing, stability assessment, and impurity profiling.[1]

Among various analytical techniques, High Performance Liquid Chromatography (HPLC) has emerged as the most widely applied method for API analysis due to its high sensitivity, selectivity, reproducibility, and versatility. HPLC allows efficient separation of compounds with diverse physicochemical properties, making it suitable for both simple and complex pharmaceutical matrices. The technique is particularly valuable in quantifying APIs in bulk drug substances, detecting related substances and degradation products, and performing stability-indicating studies under forced degradation conditions.[2]

In modern pharmaceutical quality control laboratories, HPLC methods must comply with international regulatory standards. Guidelines issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use provide harmonized requirements for analytical method validation, ensuring that developed methods are scientifically sound and globally acceptable. Similarly, regulatory authorities such as the United States Food and Drug Administration and the European Medicines Agency require validated chromatographic methods as part of drug approval dossiers and ongoing quality assurance programs.[3]

The development of an HPLC method for API analysis involves systematic optimization of chromatographic parameters including column selection, mobile phase composition, pH, flow rate, and detection wavelength. The primary objective is to achieve adequate resolution, symmetrical peak shape, acceptable retention time, and reproducible results. Following development, the method must undergo validation to demonstrate its specificity, linearity, accuracy, precision, robustness, and sensitivity in accordance with regulatory expectations.

This review focuses on the scientific principles underlying HPLC-based method development for APIs, discusses critical validation parameters as per international guidelines, and highlights the importance of validated chromatographic methods in ensuring pharmaceutical quality control and regulatory compliance.[4]

2. Overview of High-Performance Liquid Chromatography (HPLC)

2.1 Principle of HPLC

High-Performance Liquid Chromatography (HPLC) is an advanced analytical technique extensively applied in pharmaceutical quality control for the separation and quantification of Active Pharmaceutical Ingredients (APIs). The method is based on the differential distribution of analytes between a stationary phase and a liquid mobile phase under high pressure. When a sample solution is introduced into the mobile phase and passed through a chromatographic column, each component interacts differently with the stationary phase depending on its physicochemical properties such as polarity, solubility, and molecular structure.[5]

These differences in interaction result in distinct migration rates, allowing effective separation of compounds within a mixture. In pharmaceutical analysis, reverse-phase HPLC (RP-HPLC) is most commonly employed, where a non-polar stationary phase (typically C18 bonded silica) and a relatively polar mobile phase are used. Components are separated mainly on the basis of hydrophobic interactions. The separated analytes elute at specific retention times and are recorded as peaks on a chromatogram. The area under each peak is directly proportional to the concentration of the analyte, enabling accurate quantitative determination.[6]

The use of high pressure enhances resolution, reduces analysis time, and improves reproducibility, making HPLC particularly suitable for routine API analysis, impurity profiling, and stability studies.

2.2 Instrumentation of HPLC

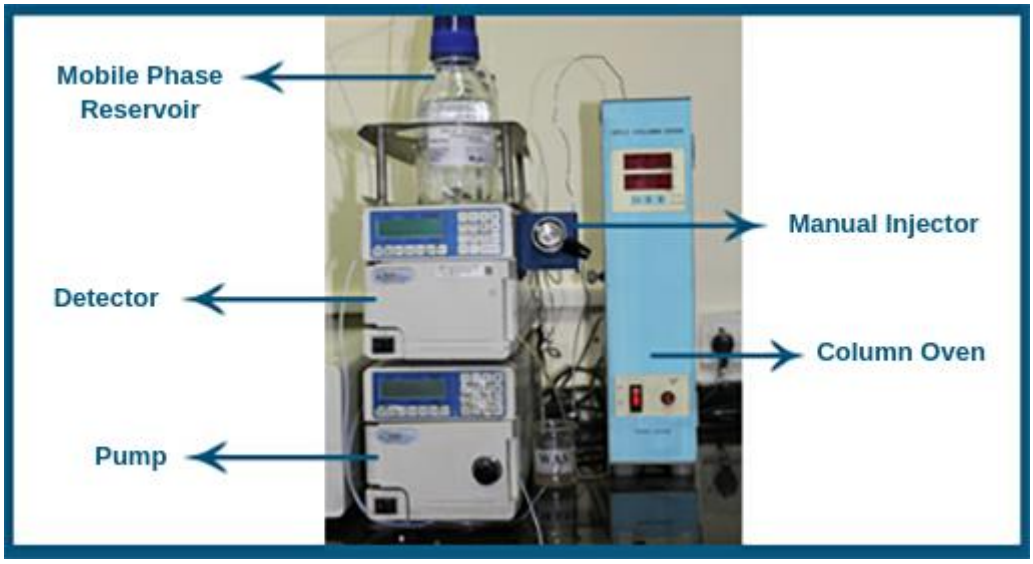

Figure 1: HPLC Instrumentation

The system begins with a solvent reservoir that contains the mobile phase. A high-pressure pump delivers the mobile phase at a constant and controlled flow rate. The injector introduces a precise volume of the sample into the mobile phase stream.[7]

The chromatographic column, packed with stationary phase particles, is the central component where separation of analytes occurs. Parameters such as column length, internal diameter, and particle size significantly influence resolution and efficiency. After separation, the analytes pass through a detector, commonly a UV or photodiode array (PDA) detector, which measures absorbance at a selected wavelength.[8]

The detector response is transmitted to a data acquisition system, where chromatograms are generated and peak parameters such as retention time, peak area, and peak height are calculated. Modern HPLC systems may also include autosamplers and column ovens to enhance precision and reproducibility.[9]

The integrated functioning of these components ensures accurate, sensitive, and reproducible analysis, establishing HPLC as a cornerstone technique in pharmaceutical API quality control.

3. Method Development of API by HPLC

The development of a robust and reliable HPLC method for the analysis of Active Pharmaceutical Ingredients (APIs) is a systematic and scientifically driven process. The primary objective of method development is to establish chromatographic conditions that ensure accurate, precise, specific, and reproducible quantification of the API in bulk drug substances and finished formulations. A well-developed method should also be capable of separating the API from impurities, degradation products, excipients, and other potential interferences. The process involves careful optimization of chromatographic parameters, guided by the physicochemical characteristics of the drug molecule such as solubility, polarity, pKa, stability, and UV absorbance properties.[10]

3.1 Selection of Column

Column selection is one of the most critical steps in HPLC method development. The stationary phase determines the selectivity and resolution of the chromatographic system. In pharmaceutical analysis, reverse-phase columns are predominantly used due to their versatility and suitability for a wide range of APIs. Octadecylsilane (C18) columns are commonly preferred because they provide strong hydrophobic interactions and good retention for moderately polar to non-polar compounds.[11]

Column dimensions, including length (commonly 150 mm or 250 mm), internal diameter (typically 4.6 mm), and particle size (3–5 µm), significantly influence separation efficiency. Longer columns generally provide better resolution but increase analysis time and back pressure. Smaller particle sizes enhance column efficiency and peak sharpness but require higher operating pressure. Therefore, selection of column specifications must balance resolution, run time, and system pressure constraints.[12]

In some cases, alternative stationary phases such as C8, phenyl, cyano, or polar embedded columns may be explored if peak tailing, co-elution, or poor selectivity is observed. Column temperature control is also essential, as it can influence retention time and peak symmetry.[13]

3.2 Selection of Mobile Phase

The mobile phase plays a decisive role in achieving proper separation and peak characteristics. It generally consists of an aqueous component (often a

buffer solution) and an organic modifier such as methanol or acetonitrile. The choice of organic solvent depends on factors such as UV transparency, viscosity, elution strength, and compatibility with the stationary phase.

Buffer selection and pH adjustment are particularly important when analyzing ionizable APIs. The pH of the mobile phase should be controlled within a range that ensures the analyte remains in a stable and consistent ionization state. Proper pH selection minimizes peak tailing and improves reproducibility. Typically, phosphate buffers are widely used due to their buffering capacity and compatibility with UV detection.[14]

The proportion of aqueous to organic phase directly influences retention time and resolution. Increasing the organic content generally decreases retention time in reverse-phase systems. Both isocratic and gradient elution modes may be applied. Isocratic elution is suitable for simple mixtures, while gradient elution is preferred for complex impurity profiling and stability-indicating methods.[15]

Table 1: Key Factors Affecting Mobile Phase Optimization in HPLC Method Development

Parameter	Impact on Separation	Optimization Strategy
Organic solvent type	Influences elution strength and peak shape	Select based on analyte polarity and UV cutoff
Buffer type	Affects pH stability and reproducibility	Choose buffer with adequate capacity
pH of mobile phase	Controls ionization and peak symmetry	Adjust near pKa of analyte
Organic phase ratio	Alters retention time and resolution	Optimize to achieve desired separation
Elution mode	Determines separation efficiency	Use gradient for complex mixtures

3.3 Selection of Detection Wavelength

Detection wavelength selection is based on the UV absorption characteristics of the API. Initially, the drug solution is scanned using a UV-visible spectrophotometer to determine the maximum absorbance wavelength (λmax). The chosen wavelength should provide high sensitivity and minimal interference from solvents or excipients.[16]

For compounds with multiple absorption maxima, a wavelength offering optimal sensitivity and baseline stability is selected. In some cases, photodiode array (PDA) detection is employed to assess peak purity and ensure specificity. The detector response must remain linear across the intended concentration range to support accurate quantification.[17]

3.4 Optimization of Chromatographic Conditions

Once the column, mobile phase, and detection wavelength are selected, further optimization of chromatographic parameters is carried out to refine system performance. Parameters such as flow rate, injection volume, and column temperature are adjusted to achieve symmetrical peak shape, acceptable retention time, and adequate resolution between adjacent peaks.

Flow rate influences both analysis time and peak efficiency. Higher flow rates reduce run time but may compromise resolution. Injection volume must be optimized to prevent peak distortion or overloading of the column. Column temperature control enhances reproducibility and may improve peak symmetry, especially for temperature-sensitive analytes.[18]

During optimization, system suitability parameters such as theoretical plates, tailing factor, resolution, and retention time consistency are evaluated to confirm acceptable chromatographic performance.

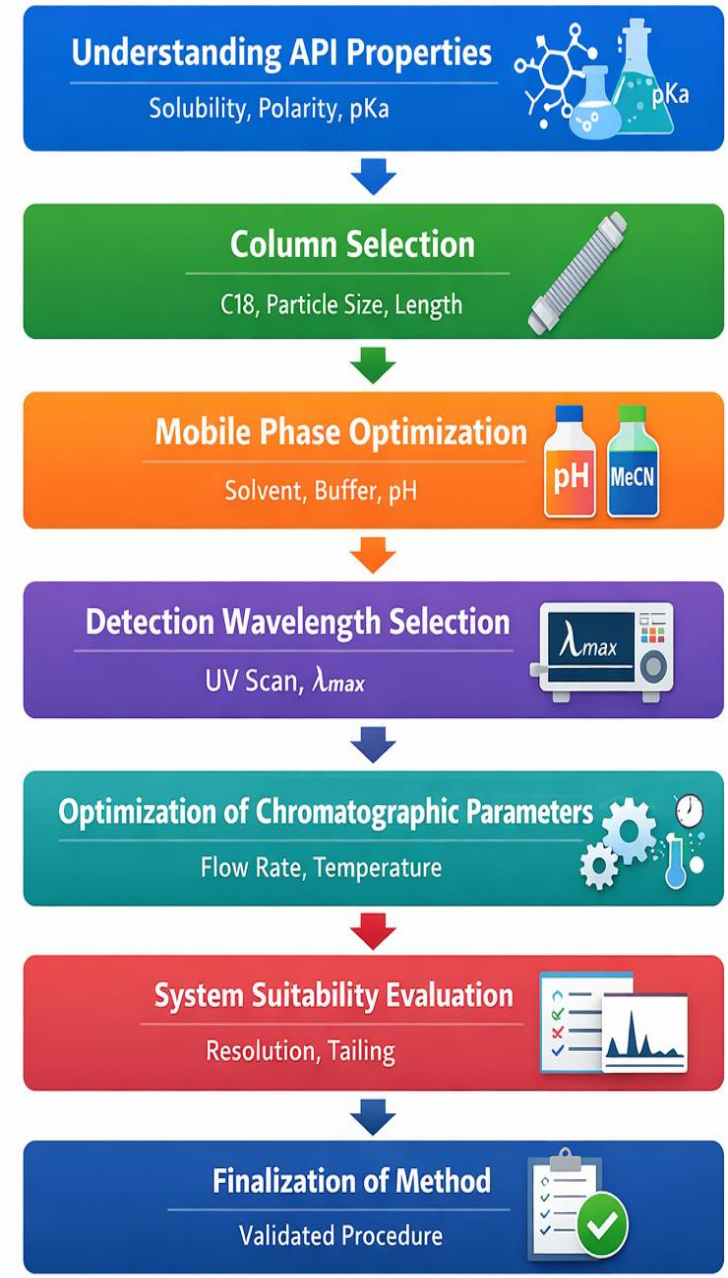

Figure 2: Flowchart of HPLC Method Development Process

3.5 Sample Preparation

Proper sample preparation is essential to ensure accurate and reproducible results. The diluent should effectively dissolve the API without causing degradation or precipitation. Compatibility between diluent and mobile phase must be maintained to avoid peak distortion.[19]

Samples are typically filtered using 0.45 µm membrane filters to remove particulate matter that could damage the column or interfere with detection. Sonication may be employed to enhance dissolution. For finished dosage forms, appropriate extraction procedures must be developed to ensure complete recovery of the API from the formulation matrix.[20]

Stability of the prepared sample solution must also be assessed to ensure that no significant degradation occurs during the analysis period. In stability-indicating methods, forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions are conducted to confirm that the method can effectively separate the API from its degradation products.[21]

4. Method Validation of HPLC Method for API Analysis

Method validation is a critical step following the development of an HPLC method for the analysis of Active Pharmaceutical Ingredients (APIs). It establishes documented evidence that the analytical procedure is suitable for its intended purpose and

capable of producing reliable and reproducible results. In pharmaceutical quality control, validated methods are essential to ensure compliance with regulatory expectations and to maintain consistency throughout the product lifecycle. Validation studies are performed in accordance with guidelines issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use under the ICH Q2 framework. These guidelines define the essential parameters that must be evaluated to confirm method performance.[22]

4.1 Specificity

Specificity refers to the ability of the analytical method to unequivocally assess the API in the presence of other components such as impurities, degradation products, and excipients. In HPLC analysis, specificity is demonstrated by ensuring that the API peak is well resolved and free from co-eluting interferences. This is typically evaluated by analyzing blank solutions, placebo mixtures, standard solutions, and sample preparations.

For stability-indicating methods, forced degradation studies are conducted under various stress conditions including acidic, alkaline, oxidative, thermal, and photolytic environments. The objective is to confirm that degradation products do not interfere with the API peak and that adequate peak purity is achieved. A method that can successfully separate the API from all potential impurities is considered specific and suitable for quality control applications.[23]

4.2 Linearity

Linearity demonstrates the method's ability to produce test results that are directly proportional to the concentration of the analyte within a specified range. During validation, multiple concentration levels of the API standard are prepared and analyzed to construct a calibration curve. The response obtained from the detector should show a consistent and proportional increase with concentration.

The linear range is selected based on the intended purpose of the method, such as assay determination or impurity analysis. A strong correlation between concentration and response indicates reliable quantification capability across the working range.[24]

4.3 Accuracy

Accuracy expresses the closeness of agreement between the value found and the true value. In pharmaceutical analysis, accuracy is commonly assessed through recovery studies by spiking known amounts of API into the sample matrix. The method should demonstrate the ability to recover the added quantity within acceptable limits.

Accuracy evaluation confirms that the method measures the true content of the API without bias. This parameter is particularly important for assay methods and content uniformity testing, where precise quantification directly impacts product quality and regulatory compliance.

4.4 Precision

Precision reflects the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. It is evaluated at different levels, including repeatability (intra-day precision) and intermediate precision (inter-day precision, different analysts, or different instruments).

A validated method should demonstrate minimal variation in results under the same operating conditions. Consistent retention times, peak areas, and calculated assay values indicate good precision. Precision ensures that routine quality control testing produces dependable results regardless of minor operational variations.[25]

4.5 Limit of Detection and Limit of Quantification

Sensitivity of the analytical method is assessed by determining the lowest concentration of the API that can be detected and quantified with acceptable reliability. These parameters are particularly important for impurity profiling and trace-level analysis.

A sensitive HPLC method ensures early detection of degradation products or low-level impurities, contributing to enhanced product safety and regulatory compliance. The selected method should demonstrate adequate signal response at low concentration levels while maintaining acceptable accuracy and precision.[26]

4.6 Robustness

Robustness evaluates the capacity of the method to remain unaffected by small deliberate variations in chromatographic conditions. During robustness testing, parameters such as mobile phase composition, pH, flow rate, column temperature, and detection wavelength are slightly altered.

A robust method maintains consistent system suitability results and acceptable assay values despite minor variations. This parameter confirms that the method is reliable for routine use in quality control laboratories, where slight environmental or operational changes may occur.[27]

4.7 System Suitability

System suitability tests are performed to verify that the chromatographic system is functioning properly before and during analysis. Parameters such as retention time consistency, theoretical plate count, resolution, and tailing factor are evaluated to ensure optimal system performance.

Table 3: Typical System Suitability Parameters

Parameter	Purpose	Acceptance Consideration
Retention Time	Confirms consistency	Should be reproducible
Theoretical Plates	Measures column efficiency	Adequate column performance
Tailing Factor	Assesses peak symmetry	Within acceptable range
Resolution	Ensures separation between peaks	Sufficient separation achieved

System suitability ensures that the analytical system is capable of generating accurate and reliable data throughout the validation and routine analysis process.

5. Applications of HPLC in API Quality Control

High-Performance Liquid Chromatography (HPLC) plays a central role in the quality control of Active Pharmaceutical Ingredients (APIs) throughout their lifecycle, from raw material testing to finished product release. Its high sensitivity, precision, and reproducibility make it an indispensable analytical tool in pharmaceutical industries operating under strict regulatory oversight.

5.1 Assay Determination of API

One of the primary applications of HPLC in quality control is the quantitative determination of API content in bulk drug substances and finished dosage forms. Assay testing confirms whether the API concentration complies with predefined specifications. The validated HPLC method ensures accurate measurement of drug potency and supports batch release decisions. Consistent assay results are critical to maintaining therapeutic efficacy and patient safety.

5.2 Impurity Profiling and Related Substances

HPLC is extensively used for detecting and quantifying process-related impurities, degradation products, and residual solvents that may arise during manufacturing or storage. Regulatory authorities such as the United States Food and Drug Administration and the European Medicines Agency require comprehensive impurity profiling data before granting marketing approval.

The technique allows separation of structurally similar impurities from the main API peak, ensuring compliance with permissible impurity limits. Stability-indicating HPLC methods are particularly valuable in identifying degradation pathways and assessing product integrity over time.[28]

5.3 Stability Studies

HPLC methods are widely applied in stability testing to evaluate the chemical stability of APIs under various environmental conditions such as temperature, humidity, and light exposure. According to guidelines issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, stability studies must demonstrate that the API maintains its quality within specified limits throughout its shelf life.

A stability-indicating HPLC method ensures that the API peak is well resolved from degradation products, enabling accurate monitoring of drug stability during accelerated and long-term studies.

5.4 Dissolution and Release Testing

In formulation development and routine quality control, HPLC supports dissolution testing by quantifying the amount of drug released from dosage forms over time. This application helps predict in vivo drug performance and ensures batch-to-batch consistency. Accurate measurement of drug release profiles is essential for regulatory submission and post-marketing surveillance.[29]

5.5 Cleaning Validation and Process Monitoring

HPLC is also applied in cleaning validation studies to confirm the absence of residual API or contaminants on manufacturing equipment. This prevents cross-contamination and ensures adherence to Good Manufacturing Practices (GMP).

Overall, HPLC remains a cornerstone analytical technique in API quality control, supporting regulatory compliance, ensuring product consistency, and safeguarding patient health.

6. Recent Advances in HPLC for API Analysis

Continuous advancements in chromatographic science have significantly enhanced the efficiency, sensitivity, and sustainability of HPLC methods used in API quality control. Modern pharmaceutical analysis increasingly integrates innovative technologies to improve resolution, reduce analysis time, and meet stringent regulatory expectations.[30]

6.1 Ultra-Performance Liquid Chromatography (UPLC)

Ultra-Performance Liquid Chromatography (UPLC) represents a major advancement over conventional HPLC systems. By utilizing columns packed with sub-2 µm particles and operating at higher pressures, UPLC achieves superior resolution, faster separation, and reduced solvent consumption. This technology allows rapid analysis without compromising accuracy or precision, making it particularly valuable in high-throughput quality control laboratories. Shorter run times improve productivity and reduce operational costs, while

enhanced peak capacity supports complex impurity profiling studies.[31]

6.2 HPLC Coupled with Mass Spectrometry (LC–MS)

The integration of HPLC with mass spectrometry has expanded the analytical capabilities of pharmaceutical laboratories. LC–MS enables not only separation but also structural identification of APIs, impurities, and degradation products. This hyphenated technique is especially useful in trace-level impurity detection and forced degradation studies. It provides molecular weight information and fragmentation patterns, assisting in the confirmation of unknown compounds. Regulatory authorities such as the United States Food and Drug Administration increasingly expect detailed impurity characterization data during drug approval processes, making LC–MS a powerful complementary tool in API analysis.

6.3 Green HPLC Approaches

Sustainability has become an important consideration in pharmaceutical analysis. Green HPLC focuses on minimizing environmental impact by reducing solvent consumption, selecting eco-friendly mobile phases, and optimizing chromatographic conditions to lower waste generation. The use of shorter columns, reduced flow rates, and less toxic organic solvents contributes to environmentally responsible analytical practices. These approaches align with global regulatory and environmental standards while maintaining analytical performance.[32]

6.4 Automation and Data Integrity

Modern HPLC systems are equipped with advanced autosamplers, column ovens, and computerized data management software. Automation improves precision, reduces human error, and enhances laboratory efficiency. Data integrity has become a critical regulatory requirement, particularly under guidelines enforced by agencies such as the European Medicines Agency. Secure electronic records, audit trails, and validated software systems ensure transparency and compliance with Good Manufacturing Practices (GMP).

Overall, recent advancements in HPLC technology have transformed API quality control by improving analytical performance, ensuring regulatory compliance, and promoting sustainable laboratory practices. These innovations continue to strengthen the reliability and efficiency of pharmaceutical analysis in an evolving regulatory landscape.[33]

7. Future Scope

The future of HPLC in API development and quality control is expected to be driven by technological innovation, regulatory harmonization, and sustainability initiatives. Emerging trends such as miniaturized chromatographic systems and microfluidic platforms may enable faster analysis with minimal solvent consumption, thereby improving efficiency and environmental compatibility. The integration of artificial intelligence and machine learning into chromatographic method development is anticipated to enhance predictive modeling, optimize separation conditions, and reduce experimental trials.

Advanced hyphenated techniques, particularly LC–MS and high-resolution mass spectrometry, will continue to strengthen impurity profiling and structural characterization of degradation products. Regulatory bodies such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use are progressively encouraging science-based and risk-based approaches to analytical method lifecycle management, promoting continuous improvement rather than one-time validation.[34]

Additionally, green analytical chemistry principles will shape future HPLC practices by emphasizing reduced solvent usage, safer reagents, and energy-efficient instrumentation. Automation, real-time release testing, and process analytical technology (PAT) integration are also expected to expand the application of HPLC beyond conventional laboratory testing into continuous manufacturing environments. These advancements collectively indicate a progressive evolution toward smarter, faster, and more sustainable API analysis.[35]

8. SUMMARY AND CONCLUSION:

High-Performance Liquid Chromatography remains a cornerstone analytical technique for the development and validation of methods used in API quality control. A systematic approach to method development ensures optimal chromatographic separation, while comprehensive validation confirms reliability, accuracy, precision, and robustness in accordance with regulatory expectations. The application of HPLC in assay determination, impurity profiling, stability testing, and process monitoring supports pharmaceutical product safety and efficacy.

With continuous advancements such as UPLC, LC–MS integration, automation, and green analytical approaches, HPLC technology continues to evolve to meet modern regulatory and industrial demands. Adherence to international standards established by organizations such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use ensures global acceptance of analytical data. Overall, validated HPLC methods play a crucial role in maintaining consistent pharmaceutical quality and protecting public health.

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