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ECO-ANALYTICAL INNOVATION IN RP-HPLC: A SUSTAINABLE FRAMEWORK FOR METHOD DEVELOPMENT AND VALIDATION

Kaur Pawanpreet*[1], Sethi Reeta[2], Singh Sudeep[3], Mehta Rachna[4], Kaur Jashanpreet[5]

[1]Research Scholar, JCDM College of Pharmacy, Sirsa.
[2]Professor, JCDM College of Pharmacy, Sirsa.
[3]Senior Manager, Walter Bushnell Research and Development Centre Pvt. Ltd, Gurugram.
[4]Associate Professor, JCDM College of Pharmacy, Sirsa.
[5]Research Scholar, JCDM College of Pharmacy, Sirsa.

***Corresponding Author: Kaur Pawanpreet**

Research Scholar, JCDM College of Pharmacy, Sirsa.

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ABSTRACT

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis for the qualitative and quantitative determination of drugs. However, conventional RP-HPLC methods often require large volumes of organic solvents, which can have adverse environmental and economic impacts. The concept of green analytical chemistry has emerged as an effective approach to minimize hazardous solvent consumption and reduce analytical waste. This review focuses on the development and validation of eco-friendly RP-HPLC methods by employing greener solvents, reducing solvent usage, and optimizing chromatographic conditions. Various aspects of method development, including selection of mobile phase, stationary phase, detection wavelength, flow rate, and system suitability parameters, are discussed. In addition, method validation parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ) according to ICH guidelines are reviewed. This review also highlights recent advances in green chromatographic technologies and various greenness assessment tools used for evaluating environmentally sustainable RP-HPLC methods. The findings indicate that eco-friendly RP-HPLC methods can provide reliable analytical performance while significantly reducing environmental impact. Therefore, green RP-HPLC represents a sustainable and efficient approach for modern pharmaceutical analysis.

KEYWORDS: Eco-friendly RP-HPLC, Green Solvent, Method Development, Method Validation, Pharmaceutical Analysis, Greenness Assessment Tools.

1. INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques for qualitative and quantitative analysis in pharmaceutical industries due to its high sensitivity, accuracy, and reproducibility. Among the various chromatographic modes, Reversed-Phase HPLC (RP-HPLC) is the most commonly employed technique because of its ability to separate a wide range of pharmaceutical compounds efficiently.[1]

Conventional RP-HPLC methods generally utilize large volumes of organic solvents such as methanol and acetonitrile as mobile phase components. Although these solvents provide excellent chromatographic performance, they are associated with several environmental and health concerns, including toxicity, hazardous waste generation, high disposal costs, and environmental pollution.[2]

Increasing awareness regarding environmental sustainability has led to the emergence of Green Analytical Chemistry (GAC), which aims to reduce the ecological impact of analytical procedures while maintaining analytical quality. The principles of GAC focus on minimizing solvent consumption, reducing waste generation, lowering energy requirements, and

replacing hazardous chemicals with safer and renewable alternatives.[3]

Eco-friendly RP-HPLC represents the application of Green Analytical Chemistry principles in chromatographic analysis. The primary objective of eco-friendly RP-HPLC is to develop analytical methods that are environmentally benign, economically feasible, and analytically robust. This can be achieved through the use of greener solvents such as ethanol and water-rich mobile phases, reduction in analysis time, optimization of flow rate, and minimization of solvent consumption.[4]

Therefore, eco-friendly RP-HPLC has become an important strategy for quantitative estimation and pharmaceutical quality control. It not only ensures reliable analytical performance but also supports environmental protection, laboratory safety, regulatory compliance, and sustainable development goals.[1,2,3,4,5,6,7,8]

1.1 Principle of Eco friendly RP-HPLC

Green Analytical Chemistry is based on the principle of reducing the environmental impact of analytical procedures while maintaining accuracy and reliability. It emphasizes the use of direct analytical techniques that minimize sample preparation and reduce the consumption of chemicals and reagents. The principles include using smaller sample sizes, performing in-situ analysis whenever possible, and integrating analytical processes to save resources. Green methods promote automation and miniaturization to decrease solvent and energy consumption. They encourage the use of safer, less toxic, and renewable solvents and reagents, such as water and ethanol, instead of hazardous chemicals. These principles help achieve sustainable, cost-effective, and environmentally responsible analytical practices.

1.2 Eco friendly Approaches for RP-HPLC

1.2.1 Solvent Selection

One of the most important factors affecting the environmental impact of HPLC is the choice of solvents. Green solvents, such as water or water combined with environmentally friendly organic solvents like ethanol and water rich mobile phases, should be preferred whenever possible. These solvents are biodegradable and have a lower environmental impact than conventional organic solvents.[9]

1.2.2 Solvent Recycling and Reuse

The implementation of solvent recycling systems can greatly reduce solvent waste and minimize the environmental footprint of HPLC analyses. Whenever practical, used solvents should be purified and reused in future analytical procedures.[10]

1.2.3. Miniaturization

The application of micro-HPLC or nano-HPLC systems can significantly decrease solvent consumption and waste production while maintaining or enhancing analytical performance. Miniaturized systems also reduce analysis time and improve energy efficiency.[11]

1.2.4 Column Selection

Selecting an appropriate HPLC column can improve separation performance and shorten analysis time, leading to reduced energy and solvent consumption. Columns with extended lifespans also help lower replacement frequency and waste generation.[12]

1.3 Green Detectors

- Environmentally Friendly Detection Systems.
- The use of eco-friendly detectors contributes to sustainable HPLC practices. For example, UV-Visible detectors are widely used and generate minimal hazardous waste and require relatively lower resources during routine analysis.
- Green Sample Preparation.
- Optimized sample preparation techniques reduce the quantity of samples and reagents required for analysis. This helps decrease waste production and lowers overall energy usage.
- Energy Conservation
- HPLC instruments often require substantial amounts of energy. Using energy-efficient equipment, switching off instruments when not in use, and optimizing operating conditions can significantly reduce power consumption.
- Green Chemistry Concepts
- Method development should incorporate green chemistry principles, including waste reduction, the use of safer chemicals, and the design of processes that maximize atom economy and sustainability.
- Analytical Quality Assurance
- Ensuring accurate and reliable analytical results minimizes the need for repeated analyses. This reduces unnecessary consumption of materials, energy, and other resources while decreasing waste generation.

1.4 Hydrotropic Reagents

Hydrotropic reagents, commonly referred to as hydrotropes, are water-soluble substances capable of markedly increasing the solubility of poorly water-soluble drugs when present in relatively high concentrations. This process is termed hydrotropic solubilization, a concept first described by Carl Neuberg in 1916. Unlike surfactants, hydrotropes do not possess a critical micelle concentration (CMC) and therefore do not solubilize drugs through micelle formation. Instead, they enhance aqueous solubility by promoting weak intermolecular interactions such as hydrogen bonding, π–π stacking, hydrophobic association, and changes in the organization of surrounding water molecules.

Hydrotropic agents are attractive for pharmaceutical applications because they exhibit excellent water solubility, chemical stability, low toxicity, biodegradability, affordability, and ease of handling. These characteristics have encouraged their widespread

use in pharmaceutical formulation development, analytical method development, dissolution studies, extraction procedures, and quality control testing. Frequently used hydrotropic agents include urea, nicotinamide, sodium benzoate, sodium salicylate, sodium citrate, sodium acetate, sodium xylene sulfonate, and sodium cumene sulfonate.

The mechanism responsible for hydrotropic solubilization is influenced by the physicochemical properties of both the drug and the hydrotropic agent. Hydrotropes increase the apparent aqueous solubility of drugs by reducing crystal lattice energy, improving drug–water interactions, and facilitating molecular association between solute and hydrotropic molecules. Importantly, this enhancement occurs without causing any chemical modification of the drug. Consequently, hydrotropic solubilization has become an effective approach in pharmaceutical analysis, particularly in UV-visible spectrophotometry and reverse-phase HPLC (RP-HPLC), where aqueous hydrotropic solutions can replace or reduce the use of hazardous organic solvents.[13,14,15,16]

Hydrotropic Reagents in Green Chemistry

Hydrotropic reagents play an increasingly significant role in green analytical chemistry by reducing dependence on environmentally hazardous organic solvents. Conventional pharmaceutical analytical methods often rely on solvents such as methanol, acetonitrile, and chloroform, which contribute to laboratory hazards, environmental pollution, and costly waste disposal. Hydrotropic systems provide an environmentally friendly alternative by utilizing water as the principal solvent while maintaining reliable analytical performance.

The incorporation of hydrotropic reagents supports several principles of green chemistry, including the use of safer solvents, prevention of chemical waste, reduction of pollution, improved energy efficiency, and development of sustainable analytical methods. Their application decreases volatile organic compound (VOC) emissions, minimizes toxic waste generation, improves laboratory safety, and lowers operational costs. Numerous pharmaceutical studies have demonstrated that hydrotropic solvent systems can achieve satisfactory sensitivity, precision, and accuracy while significantly reducing the environmental impact of analytical procedures.

1.5 Mixed Solvency

The mixed solvency concept is a modern solubility enhancement strategy based on the principle that all water-soluble substances possess some degree of solubilizing capability. Rather than depending on a single solubilizing agent at a high concentration, this approach employs a combination of two or more water-soluble solubilizers at lower concentrations to produce a greater overall increase in drug solubility. This concept was introduced by Dr. R. K. Maheshwari and has become an important technique in pharmaceutical research because it enhances solubility while minimizing toxicity and formulation-related problems.

Mixed solvency systems generally consist of combinations of hydrotropes, co-solvents, and other water-soluble excipients such as urea, nicotinamide, sodium benzoate, sodium citrate, sodium acetate, glycerol, propylene glycol, sorbitol, and polyethylene glycol (PEG 400). These components act collectively through hydrogen bonding, hydrophobic interactions, and other weak intermolecular forces to produce additive or synergistic improvements in drug solubility.

Compared with conventional solubilization techniques, mixed solvency provides several important advantages. Because each individual solubilizer is used at a lower concentration, the possibility of toxicity, irritation, precipitation, and formulation instability is considerably reduced. In many cases, the combined action of multiple solubilizers produces a greater enhancement in drug solubility than that obtained using a single solubilizing agent. The mixed solvency approach has been successfully employed for poorly water-soluble drugs including Ibuprofen, Naproxen, Aceclofenac, Ketoprofen, and Ornidazole.

From the perspective of green chemistry, mixed solvency represents a sustainable alternative to organic solvent-based analytical methods. Water serves as the primary solvent, while carefully selected combinations of hydrotropic agents and co-solvents provide the required solubilization without compromising analytical quality. This strategy reduces hazardous solvent consumption, decreases chemical waste generation, improves laboratory safety, and lowers environmental contamination.

In addition to analytical applications, mixed solvency contributes to sustainable pharmaceutical manufacturing by reducing solvent usage, lowering production costs, and minimizing occupational exposure to toxic chemicals. Owing to these benefits, the technique has found extensive application in pharmaceutical formulation development, analytical method validation, dissolution testing, stability studies, and routine quality control. Recent investigations have recognized mixed solvency as an effective and environmentally responsible approach that aligns well with the principles of green analytical chemistry.[17,18]

2.0 HPLC

High-Performance Liquid Chromatography (HPLC) is an advanced analytical technique used to separate, identify, and quantify components in a mixture. It works by forcing a liquid sample (mobile phase) through a column packed with stationary phase particles under high pressure, causing components to travel at different speeds based on their chemical properties. The separation of the sample is based on the differences in

the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase. Depending upon the partition behaviour of different components, elution at different time takes place.[19] The sample compound with the greater affinity to the stationary layer will travel slower and for a shorter distance in comparison to compounds with less affinity which travel faster and for a longer distance.[20] The High Performance Liquid Chromatography is more versatile than gas chromatography since (a) it is not limited to volatile and thermally stable samples, and (b) the choice of mobile and stationary phases is wider.[21]

Basic Components of HPLC System.

Components.

- ❖ Solvent Reservoir
- ❖ Pump
- ❖ Injector
- ❖ Column
- ❖ Detector
- ❖ Computer/Data System

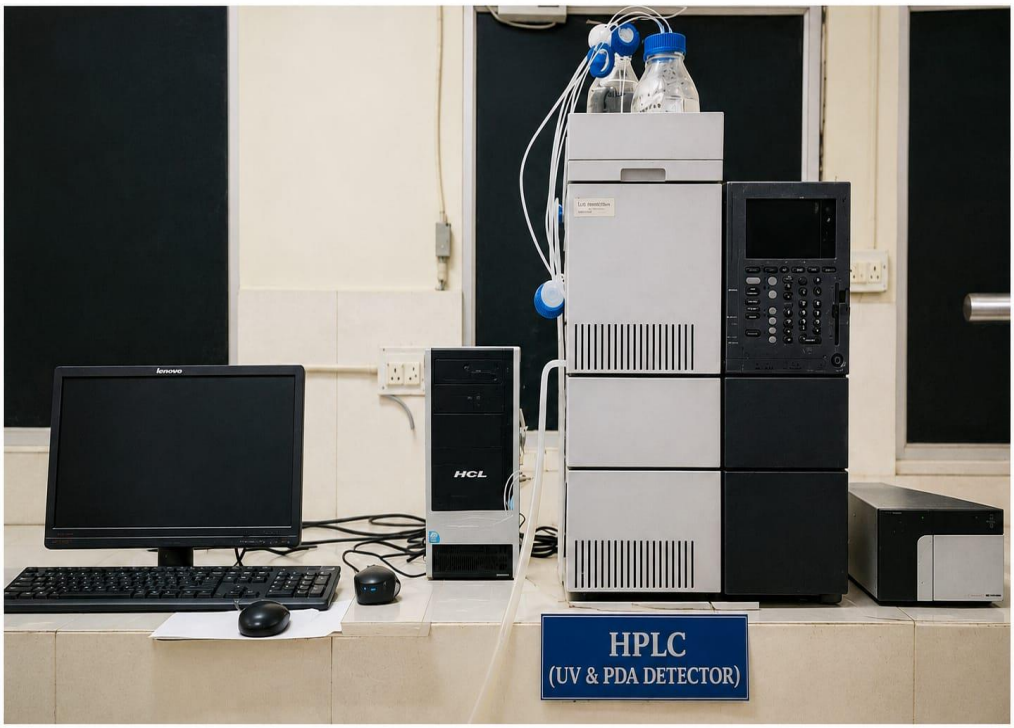

Figure 2. Instrumentation of High-Performance Liquid Chromatography (HPLC) System

Source: Laboratory HPLC Instrument

2.1 TYPES OF HPLC

2.1.1 Reversed-Phase HPLC (RP-HPLC)

Separation based on hydrophobic interactions. Non-polar analytes are retained longer, while polar analytes elute earlier.Stationary Phase: Non-polar (hydrophobic) packing, typically silica bonded with alkyl chains, such as C18 (octadecylsilane) or C8. Mobile Phase: Polar solvents (water, methanol, acetonitrile, or mixtures). Most common form, used for >70% of applications, including separating non-polar or moderately polar compounds in pharmaceuticals, such as medications. RP-HPLC is based on the principle of hydrophobic interaction.[22] In a mixture of components those analytes which are relatively less polar will be retained by the non-polar stationary phase longer than those which are relatively more polar. Therefore the most polar component will elute first.[23]

2.1.2 Normal-Phase HPLC (NP-HPLC): Separation based on polarity. Polar analytes are retained longer on the polar stationary phase than non-polar compounds. Stationary Phase: Polar packing, commonly bare silica. Mobile Phase: Non-polar organic solvents, such as hexane, chloroform, or methylene chloride. Ideal for separating polar, non-polar, and isomers that are insoluble in aqueous solvents. Separation mode in which the retention material is polar and mobile phase is nonpolar. Retained sample components are eluted in ascending order of polarity. Chemically modified silica is used as a stationary phase in this chromatography.[24] Polar compounds in the mixture that are passed through the column will stick longer to the polar silica than the non-polar compounds. Therefore, the non-polar ones will pass more quickly through the column.[25]

3.0 Green Solvents in RP HPLC

Green solvents are defined as solvents that have minimal adverse effects on human health and the environment. They are generally characterized by low toxicity, biodegradability, renewability, and reduced environmental impact. The application of green solvents in RP-HPLC is an important aspect of Green Analytical Chemistry (GAC), which aims to minimize the consumption of hazardous chemicals and reduce analytical waste while maintaining analytical efficiency and reliability.

Among the various green solvents investigated for RP-HPLC applications, ethanol has emerged as one of the most promising alternatives to acetonitrile and methanol. Ethanol is produced from renewable resources through fermentation processes, possesses relatively low toxicity, and is readily biodegradable. Water is considered the greenest solvent because it is non-toxic, non-flammable, inexpensive, and abundantly available. Modern RP-HPLC methods increasingly utilize water-rich mobile phases to reduce organic solvent consumption.[26,27]

Solvent	Toxicity	Environmental Impact	Renewability
Water	Very Low	Very Low	Excellent
Ethanol	Low	Low	Excellent
Isopropanol	Low	Low	Good
Methanol	Moderate	Moderate	Poor
Acetonitrile	High	High	Poor

4.0 Method development and validation

4.1 Study of Drug Properties

Understanding the physicochemical characteristics of the analyte is the initial step in developing an environmentally sustainable analytical method, as it helps minimize unnecessary experimentation and solvent consumption. Solubility: Evaluate the solubility of the drug in eco-friendly solvents such as ethanol, water, and ethanol–water mixtures. pKa Value: Determines the suitable mobile-phase pH to maintain the analyte in its neutral state for better retention on C18 columns or in an ionized state when required.

4.1.2 Selection of Column

The chromatographic column is the most critical component of an HPLC system and greatly influences separation efficiency. Proper column selection ensures accurate, reliable, and reproducible results, whereas an unsuitable column may lead to poor resolution and difficult interpretation. Columns are manufactured using different stationary-phase materials such as silica, polymers, alumina, and zirconium-based supports. Common reversed-phase columns include: C3 (Propyl) C4 (Butyl) C5 (Pentyl).[28]

4.1.3 Selection of Mobile Phase

The green chemistry approach focuses on replacing hazardous solvents with environmentally safer alternatives. Organic Modifier: Substitute acetonitrile and methanol with ethanol whenever possible. Ethanol is preferred because of its lower toxicity, biodegradability, and strong elution capability, allowing reduced solvent consumption. Aqueous Component: Use ultrapure water or water combined with green buffers. Green Additives: Employ acetic acid, formic acid, or ammonium acetate to control pH while avoiding toxic phosphate buffers.

4.1.4 Selection of pH

The ionization behavior of a compound is strongly influenced by the relationship between pH and pKa. The compound remains largely ionized when the pH differs significantly from the pKa value. Beyond this range, the analyte is predominantly either ionized or non-ionized, and its retention characteristics remain relatively constant.[29]

4.1.5 Selection of Detection Wavelength

The detection wavelength should be selected to maximize analyte sensitivity while minimizing interference from solvents and excipients. Recommended Range: A wavelength range of 210–230 nm is suitable for many pharmaceutical compounds, provided there is no interference from the mobile phase. Optimization: A wavelength scan in the selected diluent or mobile phase should be performed to determine the wavelength that provides maximum sensitivity.

4.1.6 Selection of Flow Rate

The flow rate should be optimized to balance analysis time, resolution, and system backpressure. An appropriate flow rate is selected by considering system pressure limits, fluid properties, and analytical requirements.

4.1.7 Temperature Optimization

Column temperature significantly influences chromatographic performance and selectivity. Increasing temperature generally reduces the viscosity of the mobile phase, lowers system backpressure, and may improve mass transfer. However, temperature changes can also alter analyte retention and selectivity, making optimization necessary to achieve the desired separation performance.[30]

4.1.8 Method optimization

Most of the optimization of HPLC method development has been focused on the optimization of HPLC conditions. The mobile phase and stationary phase compositions need to be taken into account. Optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization. To minimize the

number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined.[31]

4.1.9 Green Method Optimization

Green method optimization focuses on achieving maximum analytical efficiency with minimum environmental impact. Optimization strategies include reducing solvent consumption, shortening analysis time, lowering energy requirements, and selecting environmentally friendly reagents.

5.0 Validation

Method validations are carried out as per the ICH guidelines with the help of following parameters, respectively.

5.1 Linearity

The capacity of the analytical process to produce test findings that are exactly proportionate to the analyte concentration in the sample, within a certain range, is termed as linearity. A minimum of five distinct concentrations were prepared, and three replicates of each concentration were then made to achieve linearity.[32]

5.2 Accuracy

The accuracy of the approach determines the degree of correlation between the actual value of a measurement and its result. A comparison of test results obtained with a known value is normally employed to establish accuracy, which should be attained over the reportable range of an analytical procedure. In accordance with ICH norms, a recovery study was conducted to confirm the accuracy.[33] Accuracy is the degree to which a measurement, calculation, or specification conforms to the true or accepted standard value.

5.3 Precision

There are three precision level repeatability intermediate precision and reproducibility. Precision is the closeness of agreement among a series of measurements obtained from repeating a test on the same homogenous sample under the same conditions.[34] Precision refers to the consistency, reproducibility, and level of detail in measurements or data, indicating how close repeated measurements are to each other, regardless of the true value. Unlike accuracy, which measures closeness to the true value, high precision means small variability and high consistency, often assessed via standard deviation.

5.4 Robustness

Depending on the type of method under investigation, the evaluation of robustness should be considered during the development phase. It should show an analysis's reliability in terms of deliberate changes in technique parameters. The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, medications in method parameters and provides an indication of its reliability during normal usage.[32] Robustness is the ability of a system, method, or design to maintain performance, stability, and functionality despite perturbations, uncertainties, or environmental variations.

5.5 Specificity

The analytical technique should be specific for every stage of Development. Once all the anticipated components such as degradants, excipients/sample matrix, and sample blank peaks are identified, the method should be capable of definitively assessing the functional analyte of interest.[35] Specificity in analytical method validation is the ability to unequivocally assess the analyte (target compound) in the presence of expected components like impurities, degradants, or matrix ingredients.

5.6 Limit of Detection (LOD)

Limit of Detection (LOD) is the lowest concentration of an analyte that can be detected but not necessarily quantified under the stated experimental conditions. It represents the sensitivity of the analytical method and is generally calculated using the standard deviation of the response and the slope of the calibration curve.

5.7 Limit of Quantification (LOQ)

Limit of Quantification (LOQ) is the lowest concentration of an analyte that can be quantitatively determined with acceptable accuracy and precision. LOQ is generally higher than LOD and is an important parameter for evaluating the analytical capability of a method.

5.8 System Suitability Testing

System suitability testing is performed before sample analysis to ensure that the chromatographic system is functioning properly. Parameters such as theoretical plates, tailing factor, retention time, resolution, and peak area reproducibility are evaluated to confirm the adequacy of system performance.

6.0 Green Assessment Tools for RP-HPLC

The environmental friendliness of analytical methods can be evaluated using various green assessment tools. These tools help determine the sustainability and ecological impact of chromatographic procedures.

6.1.1 Analytical Eco-Scale

The Analytical Eco-Scale is a semi-quantitative tool used to evaluate the greenness of an analytical procedure. Penalty points are assigned for hazardous chemicals, energy consumption, and waste generation. A higher Eco-Scale score indicates a greener analytical method.

6.1.2 Green Analytical Procedure Index (GAPI)

GAPI is a comprehensive tool used to assess the environmental impact of the entire analytical process, including sample collection, preparation, reagents, instrumentation, and waste generation. It provides a

visual representation using color-coded symbols to indicate the greenness of the method.

6.1.3 AGREE Metric

AGREE (Analytical GREEnness Metric Approach) is based on the twelve principles of Green Analytical Chemistry. It provides a numerical score ranging from 0 to 1, where a higher score indicates a greener analytical procedure. AGREE offers a simple and comprehensive evaluation of analytical sustainability.

6.1.4 Analytical Method Greenness Score (AMGS)

The Analytical Method Greenness Score is a modern assessment tool that evaluates solvent consumption, energy requirements, waste production, and safety aspects of analytical methods. It assists researchers in designing environmentally sustainable chromatographic procedures

7.0 CONCLUSION

Eco-friendly RP-HPLC has emerged as an effective and sustainable analytical approach for pharmaceutical analysis. The application of Green Analytical Chemistry principles through the use of environmentally friendly solvents reduced solvent consumption, energy-efficient procedures, and waste minimization has significantly improved the sustainability of chromatographic analysis. This review highlights the important aspects of eco-friendly RP-HPLC, including green solvent selection, method development strategies, validation parameters, and greenness assessment tools.

The use of greener alternatives such as ethanol and water in place of hazardous organic solvents contributes to reduced environmental pollution, improved laboratory safety, and lower operational costs. In addition, advancements in column technology, solvent recycling systems, and miniaturized chromatographic techniques have further enhanced the applicability of green RP-HPLC methods in pharmaceutical research and quality control.

Various greenness assessment tools, including Analytical Eco-Scale, GAPI, AGREE, and AMGS, provide reliable approaches for evaluating the environmental impact of analytical procedures and support the development of more sustainable chromatographic methods.

With increasing regulatory and industrial emphasis on environmental sustainability, eco-friendly RP-HPLC is expected to play a significant role in the future of pharmaceutical analysis. Therefore, the adoption of green chromatographic practices can help achieve high analytical performance while supporting environmental protection and sustainable development goals.

Future research should focus on the development of highly efficient green chromatographic methods using renewable solvents, advanced column technologies, and artificial intelligence-based optimization tools. The adoption of eco-friendly RP-HPLC will support sustainable pharmaceutical analysis while maintaining high analytical quality and regulatory compliance.

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