



Journal of Pharmacology &amp; Drug Development

eISSN: 2958-6801



## Applications of HPLC in Pharmaceutical Analysis

Mohammed Al-shafe'a Talib <sup>\*1</sup>   Mohammed J Hamzah <sup>1</sup>  

<sup>1</sup>Department of Pharmaceutical Chemistry, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.

\*Corresponding author

Received 23 Jan, Accepted 2 May, Published 1 Jun

### ABSTRACT

High-performance liquid chromatography (HPLC) is one of the most common analytical techniques used in pharmaceutical testing and in many other scientific fields. It is widely used because it can analyze different types of materials and give accurate and reliable results. HPLC is considered an important and trusted method in many laboratories. This narrative review gives a general explanation of HPLC, including its basic principles, simple instruments, and how the technique works with different samples.

In pharmaceutical analysis, HPLC is mainly used in quality control laboratories. It is applied for drug assay, titration tests, impurity analysis, solubility studies, and stability testing. Many studies show that HPLC helps in ensuring the quality and safety of pharmaceutical products. In addition, HPLC is used in pharmacovigilance and toxicology to detect degradation products, identify counterfeit drugs, and find harmful or toxic compounds. These applications are important for protecting patient safety and supporting regulatory requirements.

HPLC is also used in other fields such as environmental analysis, forensic science, and food analysis. In environmental studies, it helps detect pollutants and trace chemicals in water and soil samples. In forensic laboratories, HPLC is used to identify unknown substances in biological or chemical samples. In food analysis, it is applied to detect additives, contaminants, and residues. Although HPLC instruments have developed over time, proper method development, validation, and correct interpretation of results are still necessary to obtain reliable data for routine laboratory work and pharmaceutical regulatory use.

**Keywords:** High-performance liquid chromatography; Pharmaceutical analysis; Pharmacovigilance; Environmental analysis; Forensic analysis; Food analysis.

### INTRODUCTION

HPLC is an essential method in chemical and pharmaceutical laboratories for analyzing substances. It effectively separates mixtures and provides quantitative results. Over time HPLC has become the tool, not just a specialized method. Analysts depend on HPLC in the analysis, where the accuracy and the consistency matter. Previous studies have reported that HPLC is a tool that can handle many kinds of analysis depending on the sample and the needed selectivity. HPLC appears in steps of drug development and testing, in science <sup>[1,7]</sup>. Researchers often use HPLC in a project to check drug purity look at how a formulation works and help create methods. HPLC also appears in quality control tests, stability studies and batch-time release analysis. Many sources say that the strong regulatory adoption of HPLC comes from the performance of HPLC and clear validation standards <sup>[3,18]</sup>. HPLC does not stay in drug analysis.

*Journal of Pharmacology & Drug Development* eISSN: 2958-6801

**How to cite:** Talib MA, Hamzah M J, Applications of HPLC in Pharmaceutical Analysis. J Pharm Drug Dev.2026: Vol 4 (1);76-91.

HPLC use has grown in years, in pharmacovigilance, environmental monitoring, criminal investigations and food analysis. The main reason, for this growth is HPLC ability to test chemicals in mixtures. When other methods do not work researchers turn to HPLC [12,30,31,32,34]. Despite the high advancements in HPLC equipment, many researchers say that the accuracy of analytical results still depends primarily on the quality of the procedure design and the analyst's understanding of it. Modern instruments can improve performance and increase accuracy, but they cannot compensate for poor chromatographic conditions. This point shows how important it is to understand the basic ideas behind chromatography when using HPLC in different types of analysis [1,9].

## HPLC Principles

The idea behind high-performance chromatography (HPLC) is simple. Performance liquid chromatography HPLC separates substances by how they interact with the phase and the mobile phase. When putting a sample into the HPLC the sample's components travel through the column at speeds. Those speed differences let the chemical compounds split apart and show up as peaks on the detector. Most analytical work treats the concept of HPLC as the rule, for every HPLC separation no matter what the sample is [2,14]. In chromatographic systems, it is well established that the stationary phase, inside the column drives the separation process. The stationary phase can interact with the analytes through forces, hydrogen bonds or hydrophobic interactions. Many sources say that choosing the phase matters more, than changing the instrument settings because the stationary phase directly changes the selectivity and the accuracy. The mobile phase moves the analytes through the column under pressure [5,22]. Many of previous articles indicate that the composition of the phase strongly affects retention time, peak shape and overall separation quality. Changes, in solvent ratio buffer concentration or pH can strongly affect the mechanism. Many studies the need for preparation and control of the phase during standardized HPLC analysis.[4,16] In HPLC separation occurs because the analytes move at rates, between the mobile phases. Chemicals that react strongly with the stationary phase stay in the column while chemicals that react less leave the column faster. The chromatography articles and reviews often talk about the concept of reaction. The concept of reaction is the reason HPLC can separate complex mixtures well [6,19]. In HPLC the high pressure pushes the phase through the columns that contain particles. Using particles increases the surface area. Makes separation work better. Using particles also needs pressure. Several sources indicate that HPLC differs from earlier liquid chromatography methods in its use of high pressure and small particles in the stationary phase [1,8,33-35].

## Types of HPLC

Different types of HPLC are used in laboratories depending on what substance is being tested and the goal of the analysis "Table 1". Analysts often do not choose a chromatographic pattern based on theoretical considerations only, but also depend on prior experience, published research, and how the molecule behaves in trial tests. Many articles show that understanding the basic differences between HPLC patterns "Figure 1" helps avoid experiment duplication when developing a procedure [6,17].

**Table 1: A Useful Comparison of the Main HPLC Modes**

HPLC mode	Strengths	Limits	Common uses
Reversed phase	Strong and flexible	Not very good for polar analytes	APIs, things that aren't pure
HILIC	Great for polar compounds	Sensitive to the situation	Drugs that are polar and metabolites
Exchange of ions	High charge selectivity	Limited use	Peptides and proteins
Exclusion by size	Not destructive	Low quality	Polymers and aggregates

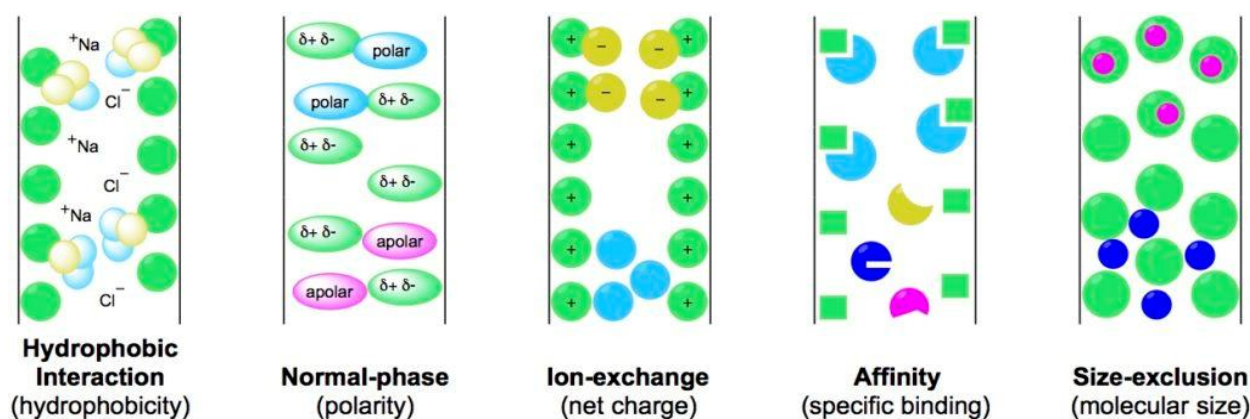


Figure 1: HPLC Separation Modes

### Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

Reversed phase HPLC (RP-HPLC) as reviewed is the most common type of HPLC used in labs. In this method, the column is nonpolar and the liquid mobile phase is polar. substances that are hydrophobic stay on the column longer, while polar substances leave faster. Many reviews shows that this stable mode makes RP-HPLC suitable for large number of tests, including titration tests, impurity identification, and solubility studies [12, 25]. But, RP-HPLC is not suitable for all materials. Highly polar or highly ionized substances may not retain anything well. In these cases, many researchers suggest using other chromatography methods instead of imposing inverted phase conditions that may negatively affect the robustness of the technique [9,18,82].

### Normal-Phase HPLC

In normal-phase HPLC, a polar stationary phase, commonly silica, is used with a nonpolar mobile phase. Adsorption interactions are the major way that separation happens. This method isn't used as much these days, though it still works for certain chemicals that don't separate well in reversed-phase systems [4,15]. One problem with normal-phase HPLC is that it is sensitive to moisture. Retention time and reproducibility might be affected by even a little quantity of water in the mobile phase. Because of this, a lot of analysts say that normal-phase procedures need to be carefully controlled in the lab and aren't very good for ordinary quality control tasks [16,28].

### Hydrophilic Interaction Liquid Chromatography (HILIC)

In recent years, HILIC has grown increasingly popular for studying polar molecules. It has a polar stationary phase and a mobile phase that is mostly made up of organic solvent, which is usually acetonitrile. Polar analytes mainly separate because they interact with a layer of water on the stationary phase. Recent HPLC review papers talk on this mechanism. Many studies indicate that HILIC offers superior retention for polar compounds compared to reversed-phase systems. However, its performance is highly sensitive to minor variations in mobile phase composition, which may limit its robustness in routine quality control applications [19,30].

### Ion-Exchange Chromatography

Ion-exchange chromatography sorts chemicals by their charge. The stationary phase features charged groups that interact with analytes that have the opposite charge. Reviews say that ion-exchange chromatography is quite beneficial when you need to separate things based on their charge differences. Small adjustments in these characteristics may cause big changes in how things are held. Because of this, a lot of sources stress the need for strict control of experimental conditions when utilizing ion-exchange chromatography for routine analysis [17,27].

### Size-Exclusion Chromatography (SEC)

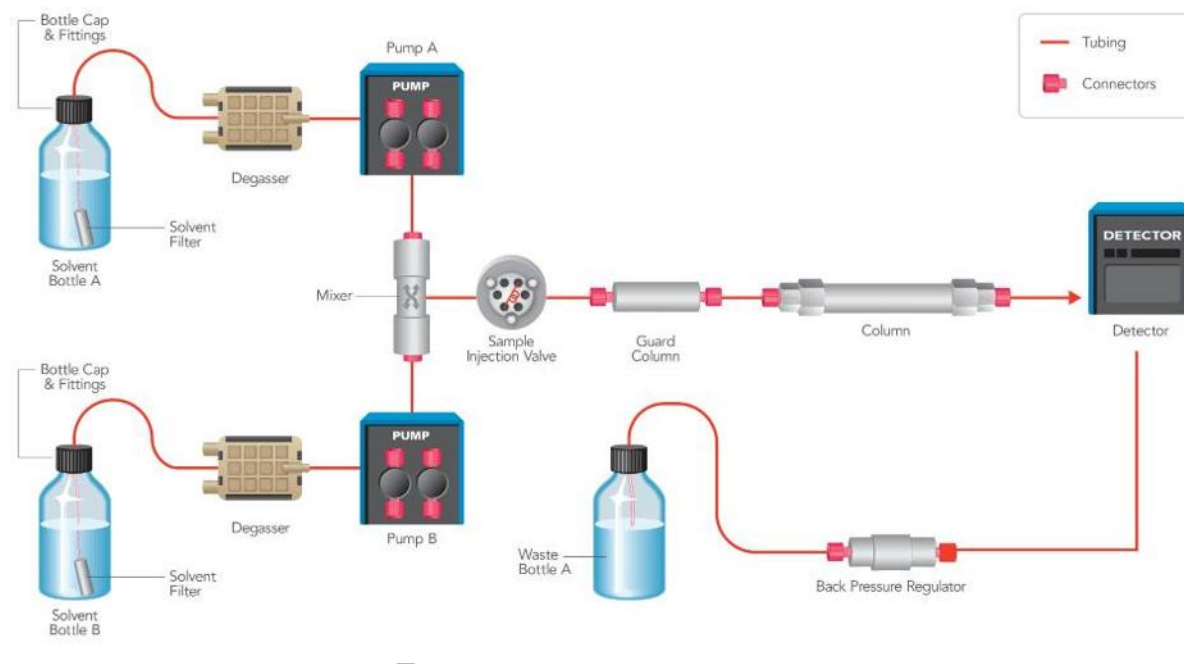
Size exclusion chromatography separates molecules based on their size, not on how they interact with each other. larger molecules are ejected first Because they can not pass through the pores of the stationary phase, followed by smaller ones. Revised chromatographic sources talk about this basic concept[5,14]. Its importance is highlighted in proteomics and stability studies, where preserving the molecule's natural structure is very important [20,29].

### HPLC Instrumentation

HPLC system consists of several components, all working together to separate and identify substances. Most modern instruments contain the same basic components, as shown in “Table 2” sometimes they are equipped with more sophisticated software as shown in “Figure 2” and automatic working systems. HPLC reviews assure that effective analytical performance depends on how efficiently these components work together, and not just on having modern equipment [2, 11].

**Table 2: The main parts of an HPLC system and how they affect how well it works.**

Part	Main role	Effect on the quality of data
<b>Pump</b>	Brings the mobile phase	Makes ensuring that retention times are steady
<b>Injector</b>	Puts the sample into the system	Affects how accurate numbers are
<b>Column</b>	Separates the items	Finds the resolution and selectivity
<b>Detector</b>	Checks the analytical signal	Affects sensitivity and specificity
<b>Software</b>	Stores and processes data	Helps keep data safe and easy to find



**Figure 2. Schematic representation of a typical HPLC system showing its main components (pump, injector, column, detector, and data system).**

## Method Development and Strength in HPLC

HPLC method development is typically performed through systematic optimization of chromatographic conditions under defined guidelines. The process usually begins with an initial method, followed by adjustments in experimental parameters to achieve optimal performance for the target analyte. This pragmatic approach has been shown to reduce development time while maintaining adequate separation and reproducibility. A key challenge in method development is achieving an appropriate balance between resolution and analysis time. While highly optimized methods may provide excellent separation, they are not always suitable for routine laboratory use due to complexity and reduced robustness. Studies in pharmaceutical analysis suggest that simple, well-established methods are often more reliable for quality control applications than highly complex approaches [47,63]. Furthermore, HPLC methods for the same drug may vary depending on the sample matrix and analytical purpose. For example, studies on azithromycin demonstrate that method conditions are frequently adjusted to accommodate different applications and sample types [87].

## Key Factors Influencing HPLC Method Performance

The selection of the stationary phase is a fundamental factor influencing HPLC method performance. C18 columns are commonly used as an initial choice due to their wide applicability and balanced retention characteristics. However, alternative stationary phases, such as phenyl or polar-embedded columns, may improve selectivity depending on the chemical properties of the analyte. Studies have shown that even minor variations in stationary phase chemistry can significantly enhance separation efficiency. In many cases, issues such as poor peak shape or co-elution can be resolved through appropriate column selection rather than modifications to instrumental parameters [60,74].

The composition and pH of the mobile phase also play a critical role in determining chromatographic behavior. These parameters directly influence analyte ionization, retention time, and peak symmetry. Maintaining stable pH conditions is particularly important for ionizable compounds, as small variations may lead to significant changes in analytical performance. Therefore, careful selection of buffer systems and strict control of mobile phase composition are essential to ensure reproducibility and consistency [45,69].

Robustness is an essential characteristic of a reliable HPLC method and reflects its ability to remain unaffected by small, deliberate variations in analytical conditions. Robustness testing typically involves evaluating changes in parameters such as flow rate, mobile phase composition, and column temperature. Studies indicate that robust methods are more suitable for routine use and facilitate method transfer between laboratories by reducing variability and minimizing the risk of analytical failure [49,66,72,79]. Additionally, well-developed RP-HPLC methods demonstrate good transferability across different laboratory settings when critical parameters are properly controlled [85].

## HPLC in the Pharmaceutical Industry

HPLC is a common in pharmaceutical labs because the HPLC can test dosage forms and give results in normal conditions. The HPLC measures the concentration of a substance and shows the profile of impurities it also checks the stability of a substance, and studies the dissolution of a substance. Studies have demonstrated that the best thing about HPLC, for pharmaceutical analysis is its flexibility. RP-HPLC-UV shown in “Figure 3” shows that HPLC procedures can be changed when the formulations or the rules change [53,68]. Modern pharmaceutical preparations contain than one component or excipient so they are complicated. When less selective methods are used that complexity makes interference likely. The literature suggests that research on applied analysis shows that HPLC can select the ingredient from the excipients and the other compounds even in the complex formulations [57,73]. Many studies report that RP-HPLC can measure isoniazid in the form and, in the preparations. The studies show linearity, precision and recovery values [83]. “Table 3” shows that the researchers use high performance chromatography (HPLC) to analyze the drugs from the therapeutic classes. Most of the methods described rely on C18 reverse-phase columns with simple mobile phases and an ultraviolet detector. These examples demonstrate the applicability of HPLC for routine assays, stability tests, and quality control of pharmaceutical products. Recent reviews have summarized various HPLC methods used to quantify azithromycin and others “Table 3” in different sample matrices, highlighting choices of mobile phases, stationary phases, and detection parameters [86].

Table 3. HPLC applications for the analysis of drugs from different therapeutic classes

Therapeutic class	Drug name	Column type	Mobile phase	$\lambda$ (nm)	Main analytical outcome
<i>Antihypertensive drugs</i>	Amlodipine <sup>[12]</sup>	C18	Acetonitrile: phosphate buffer	238	Accurate assay and good peak resolution in tablets
<i>Antihypertensive drugs</i>	Losartan <sup>[27]</sup>	C18	Methanol: water (pH adjusted)	225	Reliable quantification with good linearity
<i>Antihypertensive drugs</i>	Valsartan <sup>[33]</sup>	C18	ACN : buffer	250	Stability-indicating assay method
<i>Antihypertensive drugs</i>	Telmisartan <sup>[58]</sup>	C18	Methanol : buffer	296	Simple RP-HPLC assay for QC
<i>Renal drugs</i>	Furosemide <sup>[34]</sup>	C18	Acetonitrile: buffer	274	Successful separation from excipients
<i>Renal drugs</i>	Spirolactone <sup>[52]</sup>	C18	Methanol : buffer	242	Quantitative analysis in tablets
<i>Renal drugs</i>	Hydrochlorothiazide <sup>[66]</sup>	C18	ACN : water	270	Routine assay in combined products
<i>Cardiovascular drugs</i>	Atenolol <sup>[41]</sup>	C18	Methanol: phosphate buffer	226	Precise and reproducible assay results
<i>Cardiovascular drugs</i>	Metoprolol <sup>[60]</sup>	C18	ACN : buffer	223	Validated method for routine QC
<i>Cardiovascular drugs</i>	Digoxin <sup>[48]</sup>	C18	Acetonitrile: water	220	Sensitive determination in dosage forms
<i>Cardiovascular drugs</i>	Clopidogrel <sup>[69]</sup>	C18	ACN : buffer	235	Assay and impurity monitoring
<i>Antibiotics</i>	Azithromycin <sup>[87]</sup>	C18	Acetonitrile: buffer	210	Suitable method for different sample types
<i>Antibiotics</i>	Cephalexin <sup>[82]</sup>	C18	Methanol: buffer	262	Validated method with acceptable accuracy
<i>Antibiotics</i>	Isoniazid <sup>[83]</sup>	C18	ACN : buffer	265	Method development and validation
<i>Antibiotics</i>	Amoxicillin <sup>[71]</sup>	C18	ACN : buffer	229	Stability-indicating assay method
<i>Antidiabetic drugs</i>	Metformin <sup>[55]</sup>	C18	Acetonitrile: phosphate buffer	233	Simple and robust method for routine analysis

<i>Antidiabetic drugs</i>	Sitagliptin <sup>[61]</sup>	C18	Methanol : buffer	267	Accurate tablet determination
<i>Antidiabetic drugs</i>	Glimepiride <sup>[74]</sup>	C18	ACN : buffer	228	Quantitative analysis in dosage forms
<i>Anti-inflammatory drugs</i>	Aspirin <sup>[81]</sup>	C18	ACN : buffer	280	Assay and impurity evaluation
<i>Anti-coagulant drugs</i>	Warfarin <sup>[71]</sup>	C18	ACN : PO4 buffer	308	Therapeutic drug monitoring
<i>Anti-inflammatory drugs</i>	Diclofenac <sup>[64]</sup>	C18	Methanol : buffer	276	Stability and routine QC analysis
<i>Anti-inflammatory drugs</i>	Ibuprofen <sup>[70]</sup>	C18	ACN : water	222	Simple assay for pharmaceutical products
<i>Gastrointestinal drugs</i>	Omeprazole <sup>[68]</sup>	C18	ACN : buffer	202	Stability-indicating HPLC method
<i>CNS drugs</i>	Carbamazepine <sup>[73]</sup>	C18	Methanol : buffer	285	Quantitative tablet analysis
<i>CNS drugs</i>	Diazepam	C18	ACN : PO4 buffer	230	Bioanalytical and forensic analysis
<i>CNS drugs</i>	Paracetamol <sup>[59]</sup>	C18	ACN : water	245	Routine assay in formulations
<i>Mood stimulants</i>	Caffeine <sup>[44]</sup>	C18	ACN : buffere	272	Method development and validation studies

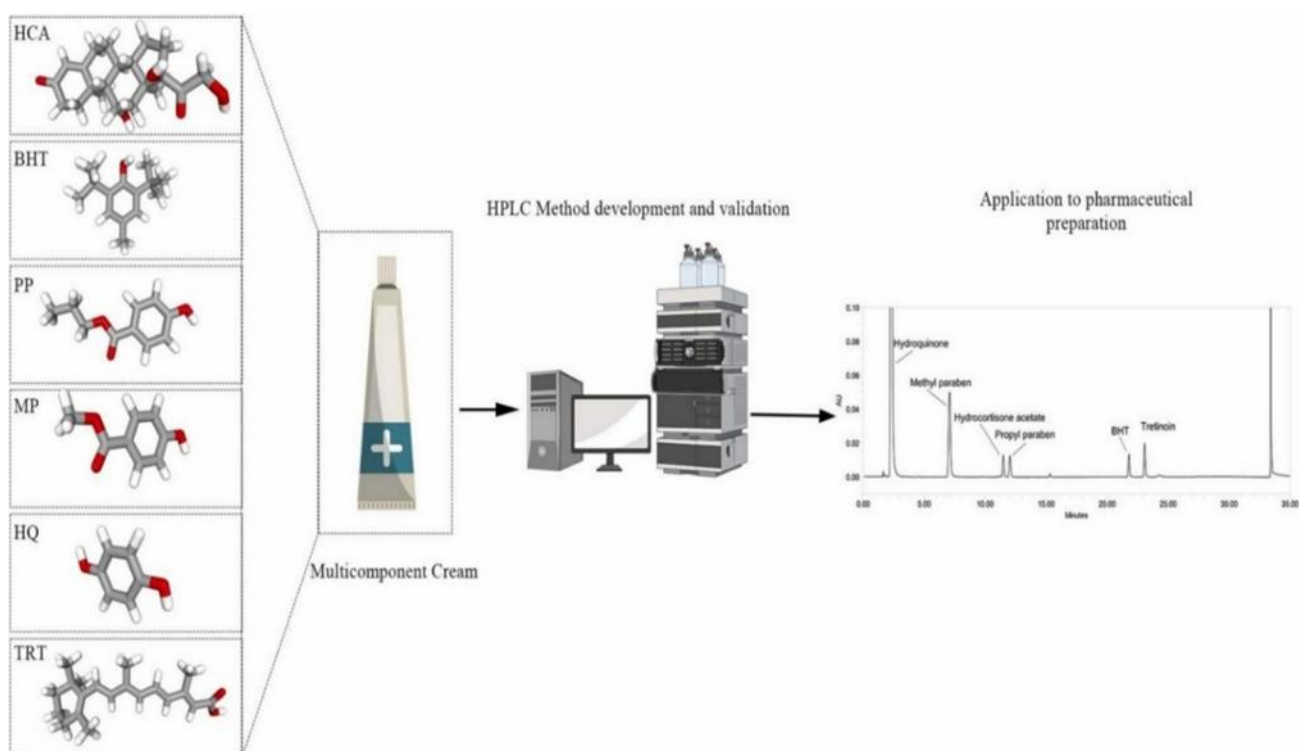


Figure 3. RP-HPLC-UV test for medicinal creams that shows stability

### **Finding out the assay**

Most reviews say that of the most typical everyday applications of HPLC in pharmaceutical quality control is assay testing. The goal is to make sure that the product has the right quantity of active medicinal ingredient. Recent reviews say that reversed-phase HPLC with UV detection is still the best method since it is easy to use, reliable, and recognized by most regulatory bodies [51,70]. Scientists are trying to speed up analyses without losing trust in the results. Instead of using the machine faster or stronger, they usually change the column or the liquid a little. These small changes help get results quicker while keeping them accurate and reliable [62,78].

### **Profiling of Impurities and Related Substances**

Impurity analysis is a job for HPLC in the medical field. Regulatory standards require the monitoring of contaminants, as they may pose significant risks to patient safety. Many studies show that impurity levels can change during growth or storage. The impurity levels need continuous monitoring. They often use stability-deficiency HPLC procedures to screen for contaminants and degradation products. These tests are done to separate the active chemicals from substances that form when materials are under stress, like heat, light, oxidation, or water. Studies show that stability tests give important information about how long products last and how to make their formulas better [64,80].

### **Testing for Dissolution**

Dissolution testing is widely recognized as it tells about how a drug leaves its dosage form and how the drug acts in the body. HPLC works better than spectroscopy for testing mixtures. Recent studies show that high-performance liquid chromatography (HPLC) based dissolution testing gives results especially for mixed products or drugs that do not dissolve well [59,75]. Many studies use dissolution testing curves made with HPLC to check any formulation changes made after approval. Analysts use these profiles to find small changes between batches that may not show up in single-point dissolution tests [56,72].

### **HPLC in Pharmaceutical Quality Control and Stability Studies**

HPLC is extensively applied in pharmaceutical quality control to ensure that products meet established regulatory standards. It is routinely used for the analysis of raw materials, in-process samples, and finished dosage forms. One of the key advantages of HPLC in this context is its ability to provide consistent and reproducible results, allowing reliable comparison between batches and over time. Consequently, quality control methods are expected to be simple, robust, and suitable for routine application rather than overly complex [61,74]. The main applications of HPLC in quality control and stability studies are summarized in "Table 4".

In addition to routine testing, HPLC plays a critical role in investigating out-of-specification results. Chromatographic data can help identify the underlying cause of unexpected findings, whether related to degradation, sample preparation, or analytical conditions. This capability supports effective root-cause analysis and corrective actions within quality systems [58,72]. While HPLC is widely applied for complex formulations, simpler spectrophotometric methods may still provide acceptable accuracy for routine assays in less complex products [84].

Stability-indicating HPLC methods are widely used to monitor changes in drug content and impurity profiles under various storage and stress conditions. These methods enable the detection of degradation products and support the evaluation of product shelf life. Studies indicate that reliable stability testing requires methods that remain consistent over extended periods and are capable of tolerating minor variations in analytical conditions. Therefore, robustness and system suitability testing are considered essential components of stability-related HPLC methods [55,80,81].

**Table 4: Uses of HPLC in Quality Control for Pharmaceuticals**

Field	Target analytes	The end result
Pharmacovigilance	Products that break down	Assessment of safety signals
Toxicology in the clinic	Drugs and their byproducts	Assessment of exposure
Surveillance after marketing	Fake drugs	Enforcement of regulations

### HPLC in Pharmacovigilance and Toxicity Assessment

Pharmacovigilance is mostly about clinical data, but analytical methods like HPLC are also very helpful. When people report bad medication responses, HPLC may be used to check the quality of the product, find degradation products, or find unexpected chemicals “Table 5”. A number of case-based evaluations show that analytical results may assist figure out whether safety problems are caused by the product or the patient [60,73]. HPLC is used to check medicines for harmful substances in samples. Getting accurate results is really important because it can affect treatment or legal decisions. Using HPLC with special chemicals makes tests more reliable and helps avoid mistakes. It also makes it easier to correctly identify substances [66,78].

**Table 5: Uses of HPLC in Pharmacovigilance and Toxicity**

Use	Goal of analysis	Impact on quality
Testing for assays	Counting APIs quantities	Release in batches
Profiling of impurities	Finding chemicals that are connected	Safety of patients
Research on stability	Keeping an eye on degradation	Setting the shelf life
Testing for dissolution	How drugs are released	Bioequivalence

### Using HPLC for Environmental Analysis

Environmental analysis has become a focus for HPLC. Environmental analysis looks at pharmaceutical, pesticide and industrial chemical residues that appear in the environment. Many of those chemicals show up in little amounts but they can still harm ecosystems and human health. Studies have shown that HPLC can handle those trace amounts and still give their numbers. Recent studies show that HPLC works well for analysis because HPLC can process samples and give quantitative results. Environmental samples such, as surface water, groundwater and wastewater have been shown overlapping chemicals, see “Table 6”. This why analysts use conditions to isolate target substances from the sample matrix. Many environmental assessments show that graded dissolution and column selection are ways to increase separation in samples. Sample variability is another problem that makes environmental analysis harder. Sample composition can change with the time of year, treatment procedures and location. Research focusing on repeated monitoring shows the need for flexible HPLC methodologies to ensure reproducibility amidst such variability [65,79].

**Table 6. HPLC Uses in the Environment**

Use	Target analytes	Goal
Testing for safety	Pesticides and mycotoxins	To check for hazardous pollutants and make sure that food items are safe to eat
Analysis of residue	Residues of veterinary drugs	To make sure that food from animals meets the maximum residual limitations set by the law
Analysis of nutrition	Vitamins and sugars	To help make sure that nutritional labels are correct and that quality is checked
Testing for authenticity	Fingerprints of chemicals	To find out whether food has been tampered with and make sure the product is real

### Forensic Uses of HPLC

In number of reviewed articles, the analytical results in forensics must be accurate and reproducible. Analysis in forensics is used in investigations. Numerous forensic studies have shown that HPLC is preferred. HPLC gives both quantity data and quantity data [63,75]. Samples are often difficult to handle because they are old, too small, or have an unknown composition. Studies in forensic toxicity show that sample preparing methods and parameters can be modified to make HPLC analysis effective in these cases. Recent research suggests that this method reduces analytical confusion and make more reliable interpretation of results in investigations [66,72].

### Food Testing and Safety Evaluation

HPLC is widely used in food analysis to keep the product safe. The food samples that were often tested in the papers contain fats, proteins, carbohydrates and natural colors so the analysis can be hard. The reviews of food analysis tells that HPLC has ability to pick out the target chemicals from the mix. Examples of food testing applications are presented in "Table 7" [64,73]. In food safety testing the HPLC method looks for pesticide residues, drugs, preservatives and food additives. The HPLC is not limited to safety testing; it also helps with food quality testing [70,78]. Analysts use data to check for contamination, compare products and verify labels. Common methods for food analysis are shown in "Figure 4". Numerous recent studies confirm that these applications help protect consumers and maintain their trust in food products [61,76].

**Table 7: Examples of HPLC Application in Food Analysis**

Use	Target analytes	Goal
Testing for safety	Pesticides and mycotoxins	To check for hazardous pollutants and make sure that food items are safe to eat
Analysis of residue	Residues of veterinary drugs	To make sure that food from animals meets the maximum residual limitations set by the law
Analysis of nutrition	Vitamins and sugars	To help make sure that nutritional labels are correct and that quality is checked
Testing for authenticity	Fingerprints of chemicals	To find out whether food has been tampered with and make sure the product is real

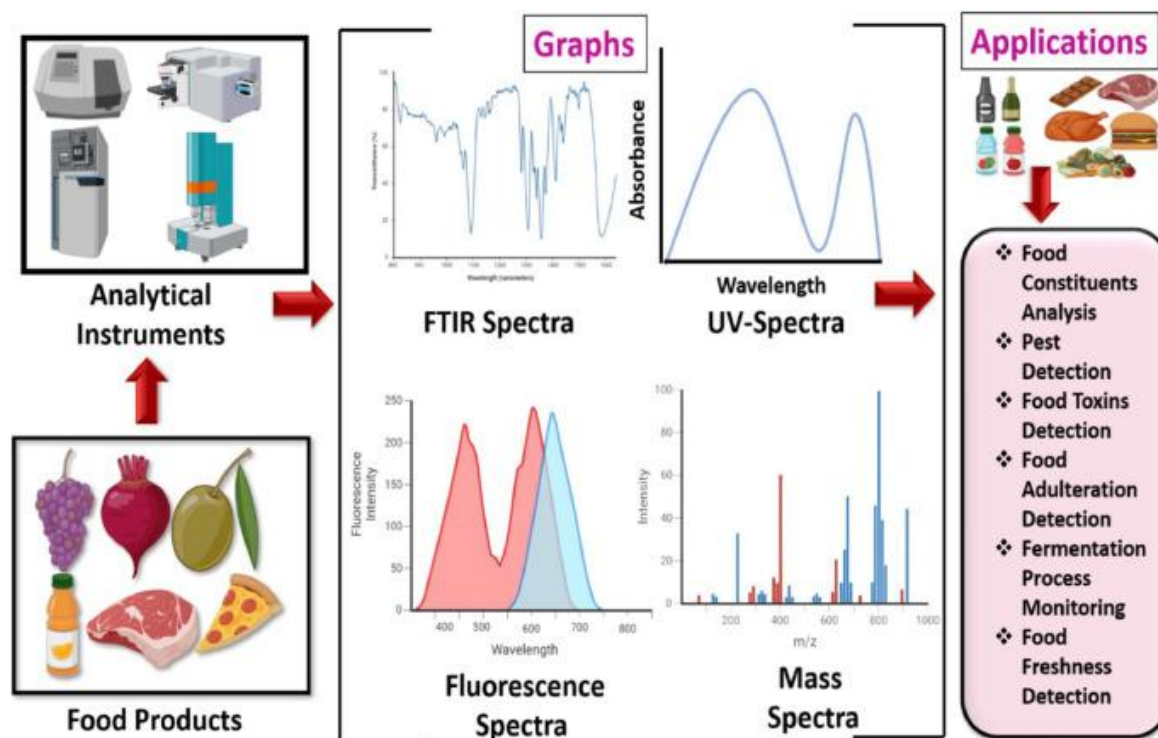


Figure 4. Common ways to analyze food.

## CONCLUSION

HPLC remains one of the most versatile and reliable analytical techniques in pharmaceutical and multidisciplinary analysis. Its ability to provide high-resolution separation, accurate quantification, and consistent performance has established it as a standard tool in quality control, stability testing, and regulatory applications. Beyond routine pharmaceutical analysis, HPLC demonstrates significant value in pharmacovigilance, environmental monitoring, forensic investigations, and food safety assessment, particularly in the detection of trace-level compounds within complex matrices. However, the effectiveness of HPLC does not rely solely on advanced instrumentation, but also on appropriate method development, careful optimization of chromatographic conditions, and proper interpretation of analytical data. While different HPLC modes offer distinct advantages depending on the analytical context, each approach is associated with specific limitations that must be considered during method selection. Therefore, achieving reliable analytical performance requires a balanced understanding of both theoretical principles and practical constraints.

Overall, this review highlights the importance of integrating method robustness, analytical reliability, and application-specific considerations to maximize the effectiveness of HPLC across various scientific fields.

## ACKNOWLEDGMENT

The Author would like to thank Al-Nahrain University /College of pharmacy Baghdad, Iraq for supporting in this research.

## CONFLICTS OF INTEREST

The author did not disclose any conflicts of interest.

## FUNDING

Non

## ETHICS STATEMENTS

Ethical approval was not required for this study, as it is based exclusively on previously published literature.

## REFERENCES

1. Snyder LR, Dolan JW. Progress in high-performance liquid chromatography: patterns and future outlook. *J Chromatogr A*. 2021;1636:461774.
2. Dong MW. New HPLC and UHPLC methods for analyzing drugs. *LCGC North Am*. 2022;40(2):18–26.
3. Kazakevich Y, Lobrutto R. The growth of chromatographic procedures in labs that are controlled. *TrAC Trends in Analytical Chemistry*. 2020;130:115964.
4. Vogt FG and Kord AS wrote a book on managing the life cycle of analytical methods in drug development. *J. Pharm. Sci*. 2021;110(2):421–430.
5. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Recent developments in the development of HPLC techniques that show stability. *J Pharm Anal*. 2020;10(2):111–122.
6. Rozet E, et al. Quality by design used in analytical procedures. *J Pharm Biomed Anal*. 2020;178:112890.
7. Reid GL and others Chromatography for checking the purity of drugs. *Pharmaceutical Technology*. 2021;45(5):30–39.
8. Danezis GP, et al. HPLC-based techniques for ensuring food safety and detecting fraud. *TrAC Trends Anal Chem*. 2021;138:116224.
9. Aronson JK. Data analysis in pharmacovigilance. *Br J Clin Pharmacol*. 2021;87(7):2771–27
10. Richardson SD and Ternes TA. Analysis of emerging pollutants using LC–MS. *Anal Chem*. 2022;94(1):102–118.
11. Pitt JJ. LC–MS in clinical and pharmacological analysis. *Clin Biochem Rev*. 2020;41(1):3–16.
12. Peters FT and Remane D. LC–MS/MS for analytical toxicology. *Analytical Bioanalytical Chemistry*. 2021;413:25–37.
13. Kümmerer K. Pharmaceuticals in the environment: analytical difficulties. *Chemosphere*. 2020;248:126013.
14. Ternes TA and others The presence of drugs in water systems. *Water Res*. 2021;189:116631.
15. Verlicchi P, et al. Pharmaceutical remnants in wastewater. *Sci Total Environ*. 740:140016.
16. Maurer HH. New developments in clinical and forensic toxicology. *Ther Drug Monit*. 2021;43(1):6–15.
17. Flanagan RJ. The function of chromatography in toxicological examination. *Clin Biochem Rev*. 2020;41(4):175–186.
18. The World Health Organization. *Pharmacovigilance: Making Sure Medicines Are Safe to Use*. WHO in Geneva, 2020.
19. Council for Harmonization in the World. *ICH Q2(R2): Checking to see whether analytical procedures are correct*. Geneva; 2022.
20. The United States Pharmacopeia. *USP <621> Chromatography*. The USP Convention will be held in 2023.
21. European Pharmacopoeia Commission. *The European Pharmacopoeia*. 11th ed. EDQM, Strasbourg, 2023.
22. Massart DL, et al. Chemometrics in chromatographic evaluation. *TrAC Trends in Analytical Chemistry*. 2020; 130:115965.
23. Brereton RG. Recognizing patterns in chromatographic data. *Analytical Techniques*. 2021;13:4203–4217.
24. New UD. Improvements in the technology of HPLC columns. *J Chromatogr A*. 2020;1625:461281.
25. Dong MW. Systems and uses of UHPLC. *LCGC North Am*. 2022;40(6):28–36.
26. Anastas PT and Warner JC wrote "Green Analytical Chemistry Perspectives." *Chemistry Green*. 23:3405–3416 in 2021.
27. Vogt FG. Chromatography and data integrity. *J Pharm Innov*. 2021;16:1–9.
28. Borman P, et al. Transferring methods and making HPLC more reliable. *J Pharm Biomed Anal*. 2020;181:113101.
29. Newton PN, et al. Low-quality drugs and analytical detection. *Lancet*. 2020;395:1985–1998.
30. Petrovic M and others LC–MS for keeping an eye on the environment. *A. Chromatogr*. 1640:461950 in 2021.
31. Nollet LML. *HPLC for Food Analysis*. 4th ed. CRC Press, Boca Raton, 2021.
32. Andersen WC and Turnipseed SB. Residue analysis in food matrices. *J Chromatogr A*. 2022;1661:462739.
33. Reid LM et al. Chromatography for checking the authenticity of food. *Food Chem*. 2021;356:129714.
34. Danezis GP and others. Using LC methods to find food fraud. *TrAC Trends in Analytical Chemistry*. 2022;146:116493.
35. Peters RJB and others Testing for drug residues in animals. *Food Addit Contam*. 2020; 37:1–14.
36. Shah VP and others Revisiting the validity of bioanalytical methods. *AAPS J*. 2021;23:56.

37. Jemal M. Bioanalysis using LC–MS/MS. *Anal Chem.* 2020; 92:705–725.
38. Dressman JB and Krämer J. *Testing for Dissolution in Pharmaceuticals.* 3rd ed. CRC Press; 2021.
39. Reid GL and others *Management of the lifecycle of chromatographic techniques.* *Pharm Tech.* 2022;46:24–33.
40. Vogt FG and Kord AS talk about QbD ideas in analytical science. *J Pharm Sci.* 2021;110:195–204.
41. Richardson SD. Advanced LC methods for analyzing water *Anal Chem.* 2021;93:124–141.
42. Ternes TA, Joss A. *Drugs in the environment.* *Environmental Science and Technology.* 2020;54:889–904.
43. Verlicchi P and Zambello E. Keeping an eye on hospital wastewater. *Sci Total Environ.* 755:142504 in 2021.
44. Maurer HH and Meyer MR. LC–MS in forensic science. *Drug Test Anal.* 2020; 12:149–162.
45. Deisingh AK. Finding fake pharmaceuticals. *Anal Bioanal Chem.* 2021;413:559–570.
46. Peters FT, et al. Hyphenated methodologies in toxicology. *Analytical Bioanalytical Chemistry.* 2022;414:607–620.
47. Brereton RG and Lloyd GR wrote "Chemometrics in Chromatography." *Analyst.* 2021;146:178–192.
48. Kromidas S. *Development of the HPLC Method.* Wiley; 2021.
49. Dong MW and Wysocki J. talk about modern gradient elution techniques. *LCGC North America* 2020;38:498–507.
50. The World Health Organization. *Making sure that drugs are safe and effective.* WHO, Geneva; 2022.
51. The European Medicines Agency. *Guideline for impurities.* EMA; 2020.
52. FDA. *Validation of analytical procedures and methods.* FDA; 2022.
53. Massart DL and Vandeginste BGM wrote a book called *Chemometrics.* Elsevier; 2020.
54. Snyder LR, Kirkland JJ, Dolan JW. *A Beginner's Guide to Modern Liquid Chromatography.* 4th edition Wiley; 2021.
55. Dolan JW. Comprehending retention and selectivity in HPLC. *LCGC.* 2020;38:56–64.
56. Kazakevich Y. Tuning selectivity in RP-HPLC. *J Chromatogr A.* 2021; 1635: 461718.
57. Hellinger R, and others HILIC uses in the analysis of drugs. *J Pharm Biomed Anal.* 2022;206:114355.
58. Pitt JJ. Tandem mass spectrometry in clinical analysis. *Clinical Biochemistry.* 2021;90:1–10.
59. Reid GL. What regulators anticipate from chromatographic data. *Pharm. Regul. Aff.* 2020;9:1000196.
60. Flanagan RJ, et al. Difficulties in toxicological analysis. *Clin Toxicol.* 2021;59:481–493.
61. Kümmerer K, et al. Environmental hazards associated with medications. *Environ.* 2022;161:107133.
62. Ternes TA. What happens to drugs in water. *Water Res.* 2020;182:116040.
63. Anastas PT. Green chemistry in analytical science. *Green Chem.* 2022;24:122–135.
64. Danezis GP. Fingerprinting based on LC in food analysis. *Control of Food.* 2020;110:107007.
65. Peters RJB. Strategies for analyzing food pollutants. *TrAC Trends in Analytical Chemistry.* 2021;136:116190.
66. Maurer HH. Understanding chromatographic data in forensic science. *Ther Drug Monit.* 2022; 44:1–9.
67. Newton PN. Keeping an eye on bad drugs. *BMJ Global Health.* 2020;5:e002505.
68. Aronson JK. Safety signals and analytical proof for drugs. *Drug Safety.* 2021;44:35–44.
69. Richardson SD. New problems in water analysis. *Anal Chem.* 2020;92:524–536.
70. Verlicchi P. Pharmaceuticals in hospital wastewater. *Sci. Total Environ.* 2022;808:152138.
71. Jemal M. Trends in bioanalytical LC–MS/MS. *Biological analysis.* 2021;13:1747–1760.
72. Brereton RG. Recognizing patterns in LC data. *Analytical Techniques.* 2020;12:440–452.
73. Nollet LML and Toldrá F. *Food Analysis by HPLC.* 2021 CRC Press.
74. Peters FT. New things in forensic LC–MS. *Drug Test Analysis.* 2021;13:6–15.
75. Kromidas S. Testing the strength of HPLC. *J Chromatogr A.* 2022;1670:462959.
76. Dong MW. Managing the lifecycle of chromatographic techniques. *LCGC North America* 2023; 41:18–26.
77. Vogt FG. Strategies for analytical control. *Journal of Pharmaceutical Sciences* 2022;111:143–152.
78. Danezis GP. Trends in finding food fraud. *TrAC Trends in Anal Chem.* 2023;154:116641.
79. Richardson SD. Improvements in environmental LC analysis. *Anal Chem.* 2023;95:102–118.
80. Snyder LR and Dolan JW talk about what the future holds for liquid chromatography. *J Chromatogr A.* 2023;1700:463308.
81. Saeed AM, Hamzah MJ, Ahmed NQ. Quantitative assay of aspirin and salicylic acid and heavy metals as impurities in Iraqi market aspirin tablets using different analytical methods. *Int J Appl Pharm.* 2018;10(5):167–172. doi:10.22159/ijap.2018v10i5.26820.
82. Saeed AM, Hamzah MJ, Mohammed OJ. Validation of liquid chromatographic analytical method for determination of cephalixin and aspirin in pure and pharmaceutical preparations. *Int J Pharm Res.* 2020;12(Suppl 1):1625–1631.

83. Merahge AH, Hamzah MJ, Al-Anbakey AM. Comparative analytical determination of isoniazid in pure and pharmaceutical preparations using spectrophotometric and RP-HPLC methods. *Biochemical and Cellular Archives*. 2019;19(2):3617–3622.
84. Al-Kaffiji Hamzzah M.J, Al-Anbakey AMS. New chromogenic reagent for the spectrophotometric determination of chlorpromazine HCl in aqueous solutions and pharmaceutical formulations. *Int J Pharm Pharm Sci*. 2013;5(Suppl 3):606–611.
85. Hamzah MJ, Alawad KM, Hameed TM, Hanna JS, Alanee RS, Najim HK, Ali RE, Qusay HL. Development of RP-HPLC method for the determination of isoniazid in pharmaceutical dosage forms. *Bull Chem Soc Ethiop*. 2026;40(1):1–9. doi:10.4314/bsce.v40i1.1
86. Taqi RMM, Hammoudy SR, Hamzah MJ. Review of HPLC Methods for Determination of Azithromycin in Different Samples. *Iraqi J Med Sci*. 2022;20(1):77–82. doi:10.22578/IJMS.20.1.10
87. Taqi RMM, Hammoudy SR, Hamzah MJ. Review of HPLC methods for determination of azithromycin in different samples. *Iraqi J Med Sci*. 2022;20(1):77–82. doi:10.22578/IJMS.20.1.10

## تطبيقات كروماتوغرافيا السائل عالية الاداء في التحليل الصيدلاني

محمد الشفيق طالب 1، محمد جاسم حمزة 1

اقسم الكيمياء الصيدلانية، كلية الصيدلة، جامعة النهرين، بغداد، العراق

### الخلاصة

تعد الكروماتوغرافيا السائلة عالية الاداء (HPLC) من اكثر التقنيات التحليلية شيوعا المستخدمة في الاختبارات الصيدلانية والعديد من المجالات العلمية الاخرى. ويعود انتشار استخدامها الى قدرتها على تحليل انواع مختلفة من المواد وتوفير نتائج دقيقة وموثوقة. وتعتبر تقنية HPLC طريقة مهمة ومعتمدة في كثير من المختبرات. يقدم هذا الاستعراض السردى شرحا عاما لتقنية HPLC، يشمل المبادئ الاساسية لها، والاجهزة البسيطة المستخدمة فيها، وكيفية عمل هذه التقنية مع عينات مختلفة. في مجال التحليل الصيدلاني، تستخدم تقنية HPLC بشكل اساسي في مختبرات السيطرة النوعية. حيث تطبق في تقدير الدواء، واختبارات المعايير، وتحليل الشوائب، ودراسات الذوبانية، واختبارات الثباتية. وتظهر العديد من الدراسات ان تقنية HPLC تساهم في ضمان جودة وسلامة المنتجات الصيدلانية. اضافة الى ذلك، تستخدم هذه التقنية في مجال اليقظة الدوائية والسموميات للكشف عن نواتج التحلل، والتعرف على الادوية المزورة، وتحديد المركبات الضارة او السامة. وتعد هذه التطبيقات ضرورية لحماية سلامة المرضى ودعم المتطلبات التنظيمية. كما تستخدم تقنية HPLC في مجالات اخرى مثل التحليل البيئي، والعلوم الجنائية، وتحليل الاغذية. ففي الدراسات البيئية، تساعد على الكشف عن الملوثات والمواد الكيميائية النزرة في عينات المياه والترربة. وفي المختبرات الجنائية، تستخدم لتحديد المواد غير المعروفة في العينات الحيوية او الكيميائية. اما في تحليل الاغذية، فتطبق للكشف عن الاضافات الغذائية، والملوثات، وبقايا المواد المختلفة. وعلى الرغم من التطور المستمر في اجهزة HPLC، الا ان التطوير الصحيح للطريقة، والتحقق من صحتها، والتفسير الدقيق للنتائج تبقى ضرورية للحصول على بيانات موثوقة للاستخدام في العمل المختبري الروتيني والتطبيقات التنظيمية الصيدلانية.

الكلمات المفتاحية: كروماتوغرافيا السائل عالية الاداء، التحليل الصيدلاني، اليقظة الدوائية، التحليل البيئي، تحليل الاغذية.