

Application of High-Performance Liquid Chromatography (HPLC) in the Analysis of Pharmaceutical Drugs

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Abstract

High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique widely used in the pharmaceutical industry to ensure the safety, efficacy, and quality of medications. Paracetamol (acetaminophen), a commonly used analgesic and antipyretic, serves as a model compound in numerous HPLC method development studies due to its prevalence in monotherapy and combination products. This review provides a comprehensive overview of the HPLC technique with specific reference to the analysis of paracetamol in raw materials and finished dosage forms. Key elements such as chromatographic principles, instrumentation, sample preparation, method validation, and modern advancements are discussed. The article also explores regulatory relevance and practical challenges encountered during the routine quality control of paracetamol-containing drugs.

Introduction

The pharmaceutical analysis of over-the-counter (OTC) drugs such as paracetamol is essential to verify label claims and detect any adulteration or degradation. As a widely consumed analgesic-antipyretic agent, paracetamol is present in numerous formulations—ranging from tablets and syrups to injectable preparations and suppositories. Its popularity in both single and fixed-dose combination therapies demands stringent quality control methods. HPLC is the preferred method due to its ability to provide accurate, precise, and reproducible results, even when paracetamol is present alongside other APIs like caffeine, ibuprofen, or codeine. This technique plays a crucial role in ensuring that the drug remains within its specified potency range throughout its shelf life and that any impurities or degradation products are well below toxic thresholds.

Principle and Components of HPLC. HPLC separates compounds based on their interaction with a stationary phase (typically C18 silica) and a mobile phase (usually a buffer-organic solvent mixture) under high pressure. When applied to paracetamol analysis, the sample is introduced via an injector and transported through the column with the mobile phase, where it is separated from other matrix components. The most common detection wavelength for paracetamol is 243 nm using a UV-Vis detector. The peak corresponding to paracetamol is then identified based on its retention time, which typically ranges between 3–6 minutes depending on the method conditions. A standard reversed-phase HPLC system includes a binary pump, autosampler, column oven, and a detector connected to chromatography software for data integration and quantification. This configuration provides high resolution and reliable quantitation, even in complex pharmaceutical matrices.

Applications in Pharmaceutical Drug Analysis. HPLC methods for paracetamol are used in a broad range of pharmaceutical applications. In dosage form assay, the technique is applied to verify the drug content per unit, which must comply with pharmacopeial standards such as USP or BP (typically 95–105% of labeled claim). For example, a validated HPLC method can be used to assess paracetamol content in 500 mg tablets across different batches. In dissolution testing, HPLC quantifies the amount of paracetamol released over time in simulated biological fluids, ensuring appropriate drug bioavailability. In combination products, where paracetamol is formulated with multiple active ingredients, HPLC enables simultaneous quantification using gradient elution or selective retention conditions. Stability studies also employ HPLC to detect and quantify degradation products such as 4-aminophenol, a known toxic impurity of paracetamol. These capabilities make HPLC indispensable for routine quality control, release testing, and regulatory submissions.

Sample Preparation and Analytical Procedure. Sample preparation for paracetamol analysis depends on the formulation type. For tablet analysis, the content of 20 tablets is typically ground and weighed to obtain a representative sample, which is then dissolved in a suitable solvent—often a mixture of water and methanol or phosphate buffer. The solution is sonicated, filtered through a 0.45 μm membrane, and diluted to the desired concentration (e.g., 100 $\mu\text{g/mL}$) using the mobile phase.

Standard solutions of paracetamol are prepared using certified reference standards and analyzed under identical conditions to construct a calibration curve. Method parameters may include a mobile phase of phosphate buffer and methanol (60:40 v/v), a flow rate of 1.0 mL/min, and a C18 column (250 mm × 4.6 mm, 5 µm). Run time is typically under 10 minutes. Peak area or height is used for quantification, and method validation parameters (linearity, precision, accuracy, robustness) must meet ICH guidelines.

Advantages and Limitations of HPLC. HPLC offers substantial advantages in the analysis of paracetamol, including high sensitivity, reproducibility, and the ability to resolve the active ingredient from excipients and impurities. It is suitable for both routine and research applications, and the method can be easily adapted to detect related substances and degradation products. For example, oxidative degradation of paracetamol can produce 4-aminophenol and N-acetyl-p-benzoquinone imine (NAPQI), both of which can be monitored using modified HPLC protocols. However, limitations include the high cost of equipment and maintenance, the need for trained personnel, and the environmental impact of organic solvents. Additionally, method development for combination products may require extensive optimization to achieve satisfactory resolution between closely eluting compounds. Despite these drawbacks, HPLC remains the method of choice for accurate and regulatory-compliant quantification of paracetamol. (Khasanova, Mamadaminova and Kholmatov, 2024)

Recent Advances and Future Perspectives. Advancements in HPLC technology have significantly improved the analysis of paracetamol. Ultra-High Performance Liquid Chromatography (UHPLC) now enables shorter run times (under 3 minutes) with improved resolution, increasing throughput in quality control labs. Coupling HPLC with tandem mass spectrometry (LC-MS/MS) enhances sensitivity and allows for detailed metabolite profiling, which is particularly useful in pharmacokinetic studies. Additionally, automation in sample injection and data analysis minimizes human error and boosts productivity. Environmentally friendly approaches, such as using ethanol or water-based mobile phases, are gaining popularity under the green chemistry initiative. Research is ongoing into microfluidic HPLC devices that offer portable, on-site testing of paracetamol in clinical and forensic settings. These

innovations are driving HPLC toward being more efficient, cost-effective, and eco-conscious, reinforcing its role in pharmaceutical quality assurance.

Conclusion. High-Performance Liquid Chromatography (HPLC) continues to be the most reliable analytical method for the quantitative determination of paracetamol in pharmaceutical preparations. Its precision, sensitivity, and compatibility with regulatory guidelines make it an essential component of modern pharmaceutical quality control. The ability of HPLC to detect impurities, monitor degradation, and ensure consistency across batches is vital for maintaining public health standards. As HPLC technology advances with integration of automation, sustainability, and high-speed analytics, its application in the analysis of paracetamol and other drugs is expected to become even more effective and widespread.

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