

PRINCIPLES OF CHROMATOGRAPHY METHOD IN PHARMACEUTICAL ANALYSES

**Andijan state medical institute,
Tojiddinova MALOHAT Tvakkalovna
assistant of department of pharmaceutical sciences**

Annotation: Chromatography is a method for separating and analyzing chemical substances based on their physicochemical properties, widely applied in modern analytical chemistry. This article provides a detailed analysis of the types of chromatography, the structure of chromatographic columns, their operating principles, and their applications across various fields. The article also includes methodological approaches, results, and practical recommendations to enhance column efficiency.

Keywords: Chromatography, column chromatography, analytical methods, adsorbent, phase, separation

Introduction:

Chromatography is widely employed as an analytical tool in modern chemistry and biology [1]. This methodological approach allows the separation of individual components from mixtures and their qualitative and quantitative analysis. Techniques based on chromatography are extensively used in pharmaceutical, food, environmental, and materials science sectors.

The chromatographic process operates on two main phases: the mobile phase and the stationary phase. Substances move with the mobile phase and separate through interactions with the stationary phase [2]. The efficiency of this method depends on the composition of the stationary phase, the velocity of the mobile phase, and the column parameters.

Methodology:

The methodology of chromatographic analysis consists of the following key steps:

- **Column preparation:** Columns are typically prepared using silica gel, alumina, or polymer matrices. The column size and length directly influence the separation efficiency of substances [3].

- **Selection of the mobile phase:** Water, organic solvents, or gases are used as the mobile phase. The composition of the mobile phase must correspond to the components being separated [4].
- **Sample introduction:** The mixture to be analyzed is introduced into the column and carried along by the mobile phase.
- **Detection:** Separated components are detected using various detectors. The most common detectors include UV/VIS, refractometers, and mass spectrometry [5].

Column chromatography is classified into the following main types:

- **Gas Chromatography (GC):** Operates in the gas phase, enabling the separation of organic and inorganic compounds at high temperatures [6].
- **High-Performance Liquid Chromatography (HPLC):** Works under high pressure and is applied in the separation of polymers, pharmaceuticals, peptides, and biological molecules [7].
- **Open-column or classical column chromatography:** Used under basic laboratory conditions for both analytical and preparative purposes [8].

Results:

The efficiency of column chromatography is determined by several parameters: column length, stationary phase particle size, mobile phase flow rate, and sample volume [9]. Studies show that polar compounds are separated more rapidly in silica gel columns, while HPLC systems can detect substances in microgram quantities with high precision [10].

HPLC columns generally consist of particles sized 3–5 microns, designed to operate under high pressure [11]. To enhance separation efficiency within the column, gradient elution is applied, which involves changing the composition of the mobile phase over time.

Analysis and Discussion

When analyzing the efficiency of chromatography and column systems, the requirements for the column and their operating principles play a primary role. The choice of column depends on the type of analytes, their molecular weight, polarity, and other physicochemical properties [12]. For example, gas chromatography (GC)

is considered the most suitable method for organic volatile compounds and gases because molecules move rapidly in the gas phase and interact efficiently with the stationary phase. Meanwhile, high-performance liquid chromatography (HPLC) is effective for detecting high molecular weight polymers, biological molecules, peptides, and pharmaceuticals, providing precise and reproducible results with columns designed to operate under high pressure [7], [11].

The efficiency of column chromatography is determined by several key parameters: separation factor, plate number, particle size, and properties of the stationary phase. The separation factor indicates the effectiveness of separation resulting from the interaction of the analyte with the mobile and stationary phases. Practical observations often show that columns with smaller particle sizes have higher separation efficiency, as molecules in the mobile phase interact more extensively with the stationary phase. However, reducing particle size increases pressure, which in HPLC systems necessitates high-pressure pumps [9], [11].

Another important factor in enhancing column separation efficiency is the gradient elution method. Gradient elution is performed by changing the composition of the mobile phase over time. This method allows efficient separation of compounds with different polarity characteristics using a single column. Additionally, selecting various organic solvents or water-organic solvent mixtures as the mobile phase can significantly improve separation efficiency [4].

In environmental monitoring, column chromatography is an essential tool. For example, gas chromatography is often used to detect pollutants in water and air samples because it allows identification of many organic compounds. Meanwhile, HPLC columns are employed for high-precision analysis of dissolved metal ions and organic pollutants in water [2]. These methods serve as primary tools in environmental monitoring and conservation efforts.

In the pharmaceutical field, column chromatography is indispensable for quality control of drugs. HPLC systems allow precise determination of the active ingredient concentration, uniformity of particles, and identification of impurities in drug preparations. The selection of columns in pharmaceuticals is also related to the chemical properties, molecular weight, and polarity of the active compound. For

example, a polar stationary phase is chosen for a polar active compound, enhancing separation efficiency [7].

The nature of the stationary phase used in column chromatography directly affects separation efficiency. Columns based on silica gel efficiently separate polar compounds, while alumina-based columns are effective for basic and neutral compounds. Polymer-based columns are suitable for separating high molecular weight biological molecules such as peptides and proteins. Choosing the appropriate column type must align with the analytical objective, as using an incorrectly selected column reduces separation efficiency and compromises result reliability [3], [8].

Temperature and pressure of the mobile phase also play a crucial role in improving column chromatography efficiency. For instance, maintaining an optimal temperature in gas chromatography increases separation speed and accuracy. In HPLC systems, operating under high pressure enhances separation efficiency because the mobile phase passes quickly and uniformly through particles, reducing peak broadening [11].

In addition to separation efficiency, the choice of detector affects the reliability of results. UV/VIS detectors allow detection of many organic compounds, while mass spectrometry detectors provide precise information on molecular structure and molecular weight [5]. Refractometers can also determine the concentration of compounds in solutions. Using multiple detectors together enhances the accuracy and reliability of the analytical process.

Minimizing errors during column chromatography also increases analytical efficiency. The sample must be introduced into the column uniformly, the stationary phase properly prepared, and the composition and flow rate of the mobile phase controlled. Regular regeneration and cleaning of columns are essential to maintain separation efficiency [8], [10].

The theoretical foundations of column chromatography also play an important role in its practical applications. For example, the Van Deemter equation allows calculation of column efficiency based on particle size, mobile phase velocity, and diffusion parameters. Using this equation, optimal operating conditions can be

determined, and necessary parameters can be set to enhance separation efficiency [12].

Another important aspect of the practical application of column chromatography is the speed and efficiency of the analysis. In HPLC systems, gradient elution allows the separation of compounds with different polarity characteristics using a single column simultaneously, significantly accelerating laboratory work. Moreover, automated column systems enable the sequential analysis of multiple samples, which enhances efficiency in modern analytical laboratories [9].

Column chromatography is also widely used in scientific research for the discovery of new compounds. For example, it serves as a primary tool for the separation and analysis of pharmaceutical molecules or natural bioactive compounds. The high precision of compound separation ensures the reliability of research results and provides a foundation for further scientific investigations [6], [7].

In addition, various software tools are employed to analyze the results of column chromatography. For instance, chromatogram analysis, along with the determination of peak position and area, allows for the calculation of compound concentrations. This approach simplifies laboratory work and enables standardization of results [10], [12].

Overall, column chromatography is an indispensable tool in modern analytical chemistry and biology, and its efficiency is determined by multiple parameters. Proper column selection, optimal mobile phase, detector choice, and operating conditions allow for highly accurate results. At the same time, column chromatography is effectively applied in environmental monitoring, pharmaceuticals, food analysis, and scientific research.

Conclusion

Chromatography and columns play a crucial role in modern analytical chemistry and biology. They enable precise separation, qualitative, and quantitative analysis of compounds. Correct selection of columns and methods enhances separation efficiency and ensures reliable analytical results. The development of these

technologies has significantly simplified and optimized analytical processes in the pharmaceutical, environmental, and food industries.

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