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Research on the Determination of Active Ingredient Content in Compound Preparations by High-Performance Liquid Chromatography

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Abstract: Compound preparations are characterized by the synergistic enhancement of multiple components, and their quality control directly affects the safety of medication and the stability of therapeutic efficacy. Due to the complex composition of compound preparations and significant differences in the physicochemical properties of various active ingredients, the detection of their content faces technical challenges, such as difficulties in separation and numerous interfering factors. High-performance liquid chromatography (HPLC), with its advantages of high efficiency, high selectivity, and high sensitivity, has become a core method for quality control of compound preparations, providing reliable data support for evaluating the stability and batch consistency of preparations. Currently, there is an increasing variety of traditional Chinese medicine compound preparations. Establishing a specific, stable, and easy-to-operate method for content determination is of great practical significance for improving the quality standards of traditional Chinese medicine and safeguarding the health of the people.

Keywords: High-performance liquid chromatography; Compound preparations; Determination of active ingredient content

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1. Introduction

Drug analysis is an important technical means to ensure drug quality, and content determination, as a core step in quality control, imposes higher requirements on the accuracy, precision, and specificity of the method. Traditional Chinese medicine compound preparations are often composed of two or more drugs, with differences in ultraviolet absorption, polarity, and chemical stability among the components, increasing the complexity of analytical detection. High-performance liquid chromatography is an effective means for analyzing complex systems and can achieve the separation of most organic drugs. When establishing the method, the properties of the chromatographic column packing, sample pretreatment methods, and system suitability should be considered to ensure that the determination results are not interfered with by excipients and have good reproducibility. It is essential to establish

a scientific method for content determination to control the quality of compound preparations throughout the entire process from research and development to production.

2. Technical optimization of HPLC for determining compound preparations

2.1. Precise construction of the chromatographic system

Optimizing the chromatographic system is the core for achieving efficient separation of multiple components in compound preparations and requires targeted design based on the physicochemical properties of the active ingredients in the preparations. In terms of stationary phase selection, the novel core-shell C18 chromatographic column, with its higher column efficiency and faster mass transfer rate, can effectively shorten separation time while improving the resolution of components with significant polarity differences, meeting the requirements for simultaneous analysis of multiple components in compound preparations [1]. For mobile phase optimization, the Quality by Design (QbD) concept should be adopted. The influence of factors such as mobile phase ratio, pH value, and flow rate on the separation effect should be investigated using response surface methodology to determine the optimal chromatographic conditions. The rational application of gradient elution mode can effectively solve the problem of peak imbalance between high- and low-polarity components in compound preparations, avoiding overlap of early-eluting peaks and peak broadening of late-eluting peaks. Precise control of column temperature is also crucial. Under constant temperature conditions, the stability of retention time can be ensured. For thermally sensitive components, low-temperature chromatographic conditions can be used to reduce component degradation and improve the accuracy of determination results. In current clinical testing, the combined application of ultra-high-performance liquid chromatography (UHPLC) and traditional HPLC has further improved separation efficiency and met the clinical demand for high-throughput testing of compound preparations.

2.2. Technological upgrades in sample pretreatment

Factors such as excipients and impurities present in compound preparations can cause matrix interference in the analysis of active ingredients, and the optimization of sample pretreatment methods directly affects the selectivity and sensitivity of the analytical method. Traditional liquid-liquid extraction methods have drawbacks such as high organic solvent consumption and low extraction efficiency. Currently, efficient pretreatment techniques such as solid-phase extraction (SPE) and magnetic solid-phase extraction (MSPE) are widely used in clinical practice. Among them, MSPE utilizes the high selectivity and simplicity of magnetic adsorbent materials to achieve rapid enrichment and purification of target substances, reducing matrix interference and organic solvent usage, which aligns with the development concept of green analysis [2]. For compound preparations containing proteins, a combination of protein precipitation and solid-phase extraction can effectively eliminate matrix interference and ensure the recovery rate of target components. During sample pretreatment, the selection of extraction solvents should be a research focus. Based on the solubility and polarity characteristics of each component, mixed solvent gradient extraction techniques can be used to improve the synchronous extraction efficiency of multiple components. At the same time, parameters such as extraction time and ultrasonic power should be optimized to reduce component loss during pretreatment and ensure the reliability and repeatability of the pretreatment method.

2.3. Collaborative optimization of the detection system

The selection and optimization of the detection system must match the detection characteristics of the active

ingredients in compound preparations to achieve accurate quantification of multiple components. Ultraviolet-visible (UV-Vis) detectors remain the primary method for routine clinical testing. Diode array detectors (DAD) can perform full-wavelength scanning, effectively distinguishing matrix interference in chromatographic peaks and improving qualitative accuracy. For components without or with weak ultraviolet absorption, detection methods such as evaporative light scattering detectors (ELSD) and charged aerosol detectors (CAD) can be used. With their advantages of a wide linear range and high sensitivity, these methods provide new approaches for the quantitative analysis of non-ultraviolet-absorbing components. The tandem application of multiple detectors is a current trend in clinical research. For example, the UV-CAD tandem detection technique can simultaneously achieve synchronous quantification of ultraviolet-absorbing and non-ultraviolet-absorbing components in compound preparations, breaking through the limitations of single-detector detection [3]. Full-wavelength scanning technology can be used to determine the maximum absorption wavelengths of each component. For components with overlapping absorption wavelengths, a multi-wavelength switching detection mode can be adopted to improve the specificity of quantification for each component. At the same time, detection parameters such as sampling frequency and bandwidth can be reasonably set to ensure the stability and accuracy of detection signals.

3. Establishment and validation of high-performance liquid chromatography (HPLC) methodology

3.1. Core elements of methodology establishment

The establishment of an HPLC analytical method must adhere to the latest technical requirements for clinical drug monitoring, ensuring both scientific rigor and applicability. It is essential to clearly define the analytes, identifying the active components to be quantified based on the compound's clinical applications and quality standards, and formulating a comprehensive analytical plan considering their physicochemical properties. The selection of reference substances should guarantee high purity and traceability, utilizing calibrated reference standards for quantification. For compounds lacking commercial reference substances, self-prepared standards should be employed after validating their structure and purity [4]. The choice of internal standards should meet criteria such as similar retention times, good resolution, and absence of matrix interference, effectively mitigating errors from injection volume variations and instrumental fluctuations to enhance quantification accuracy. During method development, attention should be paid to the synergistic optimization of chromatographic conditions and sample pretreatment methods, with multiple preliminary experiments conducted to ensure satisfactory peak shapes and resolution for all active ingredients while maintaining operational simplicity to meet routine clinical testing needs.

3.2. Key indicators for methodological validation

Methodological validation is a critical step in ensuring the reliability of HPLC methods, requiring a comprehensive assessment of key indicators (such as selectivity, linearity, precision, accuracy, and the lower limit of quantification) to comply with the latest pharmacopoeial requirements and clinical trial guidelines. Selectivity validation should consider interference from blank excipients, solvents, and other components in the formulation, ensuring a resolution greater than 1.5 between the target component peak and interfering peaks. Additionally, forced degradation studies should be conducted to investigate the impact of degradation products on quantification results, ensuring accurate quantification under component degradation conditions. Linearity testing requires at least five concentration points, with linear regression analysis performed using the least squares method, ensuring

a correlation coefficient (r) of not less than 0.999 to establish a good linear relationship between concentration and peak area [5]. Precision validation includes intra-batch and inter-batch precision, examining the consistency of multiple measurements of the same batch of samples at different times and on different instruments, with a relative standard deviation (RSD) of not more than 2.0%. Accuracy is assessed through spike recovery experiments, where reference standards at different concentrations are added to blank excipients, and the recovery rate is calculated, with an average recovery rate of 98.0–102.0% and an RSD of not more than 2.0%. The lower limit of quantification must meet the detection requirements for low-concentration clinical samples, with precision and accuracy satisfying validation requirements and a signal-to-noise ratio of not less than 10 under these conditions. Additionally, method stability, dilution reliability, and carryover can also be validated to ensure the method's stability and applicability.

4. Clinical application considerations for HPLC in compound formulation analysis

4.1. Elimination of interference from complex matrices

For solid formulations, attention should be paid to sample homogenization, employing grinding-sieving methods to ensure sample uniformity and avoid sampling errors. Simultaneously, pretreatment techniques should be used to eliminate interference from excipients such as starch, lactose, and cellulose. For liquid formulations like oral solutions and injections, the impact of excipients such as preservatives and cosolvents should be considered, with solid-phase extraction or liquid-liquid extraction methods employed to eliminate excipient interference. For oil-containing liquid formulations, emulsion breaking should be performed to improve extraction efficiency [6]. A combination of protein precipitation and ultrafiltration can be used to efficiently separate target components from protein matrices while avoiding adsorption losses of components during protein removal. In clinical applications, the matrix effect can be detected using a blank matrix spiking method. If the matrix effect is significant, matrix-matched standard curves should be used for quantification to eliminate matrix interference.

4.2. Technical adaptation for simultaneous multi-component analysis

Simultaneous multi-component analysis requires attention to the matching of chromatographic behaviors of each component, optimizing chromatographic conditions to achieve effective separation of components with different polarities and molecular weights within a reasonable analysis time, avoiding peak overlap and co-elution. For compound formulations with significant physicochemical property differences, such as coexisting acids and bases, ion-pair chromatography techniques can be utilized by adding ion-pair reagents to the mobile phase to regulate the retention behavior of each component in the mobile phase and improve separation efficiency [7]. Gradient elution can be employed to gradually adjust the proportion of the organic phase in the mobile phase, achieving sequential elution of high- and low-polarity components while controlling the gradient slope to avoid peak broadening and reduced resolution. In terms of the detection system, a multi-wavelength switching detection method can be adopted, combining the detection characteristics of different components to achieve simultaneous quantification of multiple components. Additionally, attention should be paid to the matching of linear ranges for each component, establishing reasonable concentration gradients to ensure quantification within the respective linear ranges for each component.

4.3. Integration with clinical drug monitoring

In clinical drug monitoring, it is necessary to measure the in vivo concentrations of each component in compound

formulations and study their metabolic patterns and interactions within the body. Given the low component content and complex matrix of biological samples, solid-phase extraction techniques can be used to enrich target components, improving analytical sensitivity. Simultaneously, internal standard quantification can be employed to eliminate interference from biological matrices [8]. In clinical drug monitoring, method rapidity is crucial. By optimizing chromatographic conditions, analysis time can be shortened to meet the demands of rapid clinical detection while ensuring method accuracy and precision, providing reliable blood drug concentration data for clinical dose adjustment and efficacy assessment. Furthermore, HPLC can be used for in vitro dissolution studies of compound formulations, examining the dissolution rates and velocities of each component under different dissolution conditions and analyzing the interactions between component dissolutions to provide a scientific basis for the rational use of compound formulations.

5. Development trends and clinical prospects of HPLC methods

5.1. Technological innovation and integrated development

Ultra-high-performance liquid chromatography (UHPLC), leveraging its small particle size and high-pressure characteristics, enables rapid and efficient separation of multiple components in complex systems, reducing analysis time by over 50% compared to traditional HPLC and meeting the demands of high-throughput clinical testing. By integrating the separation capabilities of HPLC with the high selectivity and sensitivity of mass spectrometry, high-sensitivity qualitative and quantitative analysis of trace and similar components in complex compound formulations can be achieved, providing an advanced technological tool for clinical analysis of complex compounds [9]. Additionally, the integration of HPLC with artificial intelligence (AI) technologies facilitates intelligent optimization of chromatographic conditions, enabling rapid screening of optimal conditions. Furthermore, AI can be employed for automatic identification and integration of chromatographic peaks, reducing human errors and enhancing analytical efficiency.

5.2. Green and automated development

Green chromatography techniques, such as supercritical fluid chromatography and hydrophilic interaction chromatography, are adopted to reduce the usage of organic solvents. Green extraction solvents and liquid-phase mobile phases are utilized to minimize environmental pollution caused by the methods. In sample pretreatment, magnetic solid-phase extraction and dispersed solid-phase extraction methods significantly reduce organic solvent consumption and enhance sample pretreatment efficiency. The implementation of fully automated sample pretreatment systems and fully automated injection systems enables complete automation of the entire process from sample pretreatment to analysis, reducing human errors and improving method reproducibility and analytical efficiency to meet the demands of testing large volumes of clinical samples.

5.3. Expansion and deepening of clinical applications

In formulation development, HPLC can be employed for prescription optimization and process screening of compound formulations, as well as for studying the content and stability of active ingredients under different dosage forms and process conditions, providing data support for formulation development [10]. By integrating HPLC with therapeutic drug monitoring (TDM) techniques, real-time monitoring of blood drug concentrations of each component in compound formulations can be achieved, providing accurate data for clinical medication.

Simultaneously, analyzing the interactions among components in vivo offers a basis for rational clinical drug use. Moreover, HPLC plays a crucial role in quality control of traditional Chinese medicine compound formulations, enabling simultaneous quantification of multiple marker components and promoting the standardization and modernization of compound quality.

6. Conclusion

In summary, HPLC plays an irreplaceable role in the analysis of active ingredients in compound formulations, accurately reflecting the quality status of the formulations and providing a technological foundation for optimizing the production process and quality evaluation. With the continuous advancement of modern analytical techniques, the emergence of new technologies such as UHPLC and hyphenated techniques will further enhance analytical efficiency and detection capabilities. In the future, emphasis should be placed on method transferability and standardization research to enhance the comparability of data across different laboratories, promote the expansion of quality control to the entire process and multiple dimensions, and provide technological guarantees for the safe and efficient use of compound formulations.

Disclosure statement

The author declares no conflict of interest.

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