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The Critical Role of Impurity Analysis in Quality Control During Drug Testing and Its Optimization Paths

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Abstract: Impurity analysis in drug testing plays a crucial role. It can identify and control harmful impurities, ensuring drug purity, stability, and safety while guaranteeing efficacy. Currently, various techniques and methods are employed for impurity analysis, including chromatographic techniques such as ion chromatography, gas chromatography, and high-performance liquid chromatography, as well as mass spectrometry techniques like liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry, and spectrophotometric methods like UV-visible spectrophotometry. However, impurity analysis techniques face challenges, including the need to improve sensitivity and accuracy, overcome difficulties in analyzing complex sample impurities, and address method reproducibility issues. Optimizing impurity analysis involves introducing advanced techniques like ultra-high-performance liquid chromatography and chromatography-mass spectrometry, establishing standardized processes, strengthening personnel training, and utilizing artificial intelligence and automation technology. These efforts enhance drug quality control levels, ensuring safe and effective medications.

Keywords: Drug testing; Impurity analysis; Chromatographic techniques; Mass spectrometry

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1. Introduction

Pharmaceuticals are special commodities that maintain human health, and their quality control is paramount. High-quality drugs ensure patients receive the expected therapeutic effect, aiding in recovery and pain relief. Poor-quality drugs may render treatment ineffective, delaying patient recovery and increasing suffering and economic burden. In severe cases, they can cause serious adverse reactions, even life-threatening situations such as anaphylactic shock due to excessive impurities. Drug quality control also impacts the medical system's credibility and effectiveness. High-quality drugs enhance patient trust in the medical system, promote the healthy development of the pharmaceutical industry, protect people's health rights, and serve as the cornerstone of a solid public health system.

2. The critical role of impurity analysis in drug testing

2.1. Identification and control of harmful impurities

In drug testing, impurity analysis is a key method for identifying and controlling harmful impurities. Through advanced analytical techniques like high-performance liquid chromatography, gas chromatography, and mass spectrometry, various impurities in drugs, including organic impurities, inorganic impurities, and residual solvents, can be precisely detected. Once harmful impurities are identified, corresponding control measures can be formulated based on their properties and sources. Starting from the source, strict control of raw material quality, optimization of the production process, and reduction of impurity generation are essential [1]. Monitoring should be strengthened during the production and storage processes to prevent impurity levels from exceeding standards. For harmful impurities already produced, methods such as purification and refinement can be employed to ensure impurity content in drugs remains within safe limits, guaranteeing drug safety and preventing adverse reactions and potential safety hazards for patients, allowing them to use medications safely.

2.2. Ensuring drug purity and stability

Drug purity and stability are important indicators of its quality, and impurity analysis plays a crucial role in this regard. The presence of impurities can directly affect the purity of drugs, reducing the content of active ingredients and affecting the treatment effect. Impurity analysis can accurately determine the types and content of impurities in drugs, ensuring that the purity of the drugs meets the standard requirements. In terms of stability research, impurity analysis can monitor the generation of impurities under various conditions such as temperature, humidity, and light. If an increase in impurities is detected, it indicates poor drug stability, and adjustments to the production process, packaging, or storage conditions may be necessary to inhibit the generation of impurities. Impurity analysis can also help researchers understand the degradation pathways and mechanisms of impurities, providing a scientific basis for drug stability and ensuring that drugs maintain stable quality during their shelf life, allowing patients to obtain reliable treatment effects.

2.3. Ensuring drug safety and effectiveness

The impact of drug impurity analysis on safety and effectiveness differs significantly between chemical drugs and biological products. In chemical drugs, even trace amounts of genotoxic impurities (such as nitrosamines) can cause DNA damage and pose a carcinogenic risk. Heavy metal residues (such as lead and arsenic) can accumulate in the body, posing a threat to kidney and liver safety. Residual solvents (such as toluene) may change the crystal form of the drug, affecting its dissolution rate and bioavailability. The core risks of biological products lie in process-related impurities: host cell proteins may trigger an immune response, neutralizing drug activity; nucleic acid residues have potential tumorigenicity; and misfolded protein aggregates not only reduce efficacy but may also enhance immunogenicity. Regarding effectiveness, degradation impurities in chemical drugs (such as oxidation products) may compete with the API for metabolic enzymes, while glycosylation variants in biological products may alter pharmacokinetic properties. Modern analytical techniques such as LC-MS/MS and CE-SDS can accurately identify different impurities. Through the QbD concept, control strategies can be established to achieve ppm-level control of chemical drug impurities and ng/mg-level monitoring of host proteins in biological products, balancing safety thresholds and therapeutic activity.

3. Current status of impurity analysis techniques and methods

3.1. Application of chromatographic techniques in impurity analysis

Chromatographic techniques achieve separation based on differences in the distribution coefficients of different substances between the mobile and stationary phases. Ion chromatography, with its unique ion exchange principle, has been widely used in detection in fields such as food, environment, and biomedicine. Compared to traditional chromatography, ion chromatography exhibits significant advantages in detecting hydrophilic components such as organic acids and bases, and is also suitable for detecting complex organic molecules such as sugars and amino acids. Therefore, ion chromatography is often used for the inspection of active ingredients in complex drug raw materials or pharmaceutical preparations [2]. In drug impurity analysis, ion chromatography is mainly used to detect inorganic ions, organic acids, and their salts; gas chromatography is adept at analyzing volatile organic substances such as pesticide residues; and high-performance liquid chromatography, with its high resolution, sensitivity, and automation, is widely used to separate complex mixtures. However, chromatographic techniques also have limitations, such as complex sample pretreatment and high detection costs. Ion chromatography and gas chromatography play important roles in specific fields, while high-performance liquid chromatography, with its multi-mode separation capability, occupies an important position in the field of drug impurity analysis.

3.2. Application of mass spectrometry in impurity analysis

Mass spectrometry, which analyzes substances by measuring the mass-to-charge ratio (m/z) of molecular or atomic ions, plays a central role in the identification of pharmaceutical impurities. High-resolution mass spectrometry (HRMS), utilizing time-of-flight or Orbitrap analyzers, offers a resolution exceeding 100,000 and a mass accuracy below 1 ppm. This enables precise differentiation of impurities with similar molecular weights, such as isomers or homologues. For instance, in detecting nitrosamine genotoxic impurities in valsartan, HRMS achieves accurate qualitative and quantitative analysis at the ng/g level through precise mass matching and isotope abundance ratio analysis, overcoming the limitations of traditional mass spectrometry in identifying trace impurities. Combined with liquid/gas chromatography, HRMS not only resolves UV-nonresponsive impurities (e.g., N-nitrosodimethylamine) but also derives unknown impurity structures through multistage fragment ion analysis, significantly enhancing the reliability of trace impurity identification in complex matrices and providing a higher-dimensional technical guarantee for pharmaceutical impurity profiling control [3].

3.3. Other commonly used impurity analysis techniques

Techniques such as high-performance liquid chromatography and gas chromatography are also crucial in impurity analysis. High-performance liquid chromatography, based on the interaction between compounds in solution and the stationary and mobile phases, enables the separation, identification, and quantitative analysis of chemical components in pharmaceuticals, making it suitable for analyzing complex mixtures. Gas chromatography, with gas as the mobile phase, is primarily used for detecting pesticide residues like organochlorines. UV-visible spectrophotometry measures the absorbance of a drug at a specific wavelength, allowing for quantitative and qualitative analysis, and is often used to determine the maximum absorption wavelength or to identify drugs through UV spectra.

4. Challenges and limitations of impurity analysis techniques

4.1. Improving analytical sensitivity and accuracy

In pharmaceutical impurity analysis, enhancing sensitivity and accuracy remains a pressing issue. On one hand, some impurities exist at extremely low levels, such as genotoxic impurities, which may be present at parts per billion concentrations, posing significant challenges to detection techniques [4]. Existing techniques like conventional high-performance liquid chromatography may not achieve the required sensitivity when detecting these impurities. On the other hand, the complexity of pharmaceutical components and severe matrix interference can also affect analytical accuracy. In complex sample analysis, two-dimensional chromatography (GC×GC or LC×LC) greatly improves resolution through orthogonal separation, but it suffers from high instrument costs and complex maintenance. Online purification techniques (e.g., online SPE) can reduce matrix interference with lower equipment investment but require optimization of purification steps. Supercritical fluid chromatography (SFC) offers high separation efficiency and low operating costs for non-polar impurities but has limited applicability. In contrast, conventional chromatography-mass spectrometry techniques (e.g., LC-MS) combine separation with high sensitivity, resulting in a higher overall cost (equipment + maintenance) but superior throughput and data reliability. Additionally, to balance economy and resolution, online purification combined with conventional chromatography or SFC can serve as an intermediate solution, while high-precision studies prioritize two-dimensional chromatography or hyphenated techniques [5]. Developing new analytical methods and technologies, such as hyphenated techniques that combine the advantages of multiple technologies like liquid chromatography-mass spectrometry, can also enhance the sensitivity and accuracy of impurity analysis in complex samples.

4.2. Difficulties in impurity analysis of complex samples

The analysis of impurities in complex samples faces numerous challenges. In complex systems such as traditional Chinese medicines (TCM) and biological products, the variety of components is extensive, and the types and sources of impurities are more intricate. Impurities in TCM may originate from various stages such as cultivation, processing, and storage of medicinal herbs, including pesticide residues, heavy metals, and mycotoxins. Biological products, on the other hand, may contain impurities like host cell proteins and nucleic acids. The physicochemical similarity between these impurities and the active ingredients of the drug increases the difficulty of separation and identification. Additionally, the matrix effect of complex samples cannot be ignored. Large molecules, ions, and other components in the matrix can interfere with instrument detection, leading to signal suppression or enhancement and affecting the accuracy of impurity quantification. For instance, in biological products, high concentrations of proteins may adsorb onto chromatographic columns, affecting their separation performance and, consequently, the results of impurity analysis. To address these issues, two-dimensional chromatographic techniques can be employed to enhance peak capacity and resolution. For example, in the impurity analysis of teicoplanin, 2D-UPLC-Q/TOF-MS is used in combination with desalting treatment after one-dimensional chromatographic separation to determine the structure of impurities [6]. Alternatively, supercritical fluid chromatography, which offers significantly higher efficiency compared to traditional liquid chromatography, can be utilized. For instance, in the separation of flavonoids from *Astragalus membranaceus*, a supercritical CO_2-methanol mobile phase is employed, and optimizing the modifier ratio (such as 13.64% entrainer) can improve the purity of paeoniflorin and albiflorin (94.11% and

85.65%, respectively) [7].

4.3. Issues with method reproducibility

Impurity analysis methods face reproducibility challenges. Variations in results can occur across different laboratories, operators, instruments, and even when using the same instrument at different times for the same impurity analysis. These discrepancies may be attributed to factors such as instrument stability, operational consistency, and sample uniformity. For example, in high-performance liquid chromatography (HPLC) analysis, batch differences in chromatographic columns, preparation errors in the mobile phase, and inaccuracies in sample injection volumes can all lead to variations in impurity peak areas or retention times, affecting method reproducibility. Similarly, in mass spectrometry, factors like instrument calibration status, ion source temperature, and mass spectrometry parameter settings can influence the stability of detection results. To enhance method reproducibility, it is essential to establish standardized operating procedures that strictly regulate instrument usage, maintenance, and calibration, ensuring instrument stability and consistency. Additionally, training laboratory personnel to improve their operational skills and adherence to protocols is crucial [8]. The use of reference materials for quality control, comparison, and calibration of analysis results can further ensure consistency across different laboratories and time points.

5. Effective paths for optimizing impurity analysis

5.1. Introducing advanced technological means

Ultra-high-performance liquid chromatography (UHPLC) technology has significantly accelerated analysis speed due to its use of smaller particle packing materials. For complex samples, this technology can accurately complete impurity analysis in a shorter time, representing a qualitative leap in analysis efficiency compared to traditional methods. The combination of chromatography and mass spectrometry, particularly liquid chromatography-mass spectrometry (LC-MS), is an innovative approach that seamlessly integrates the efficient separation capabilities of chromatography with the precise identification strengths of mass spectrometry. In the analysis of drug metabolites, for instance, LC-MS allows for the simultaneous and efficient detection of multiple components, greatly enhancing the comprehensiveness and accuracy of the analysis. On the other hand, gas chromatography-mass spectrometry (GC-MS) excels in pesticide residue detection, demonstrating high sensitivity and specificity in accurately identifying trace pesticide residues. These advanced technologies, with their outstanding characteristics, effectively overcome the challenges of impurity analysis in complex samples, providing solid and reliable data support for drug quality control and ultimately ensuring the safety and effectiveness of drugs in a comprehensive manner.

5.2. Establishing standardized work processes

In drug impurity analysis, standardized processes should span both intermediate and final product control, forming a closed-loop quality control system. Intermediate control involves setting standardized detection points at critical production nodes (such as before concentration of traditional Chinese medicine extracts or after purification steps in biological products). This includes specifying sampling methods, testing items (e.g., residual solvents or host protein content in intermediates), and thresholds. For example, HPLC can be used for rapid screening of pesticide residues in traditional Chinese medicine intermediates, while ELISA can dynamically monitor host DNA in biological fermentation broths, allowing for real-time interception of batches exceeding limits. Final product control, based on regulations such as ICH Q3, involves establishing standardized testing procedures that cover both known and unknown impurities. For instance, LC-MS/MS combined with toxicity databases can be employed for impurity profiling in finished traditional Chinese medicines, while two-dimensional chromatography coupled with high-resolution mass spectrometry can identify trace amounts of host proteins in biological products. Concurrently, mandatory stability studies (including accelerated and long-term testing) should be conducted to monitor impurity growth trends during storage. Lastly, standardized analytical methods (such as unified chromatographic column models and mass spectrometry parameters) should be shared across both intermediate and final product control stages, with the adoption of automated equipment and information systems (LIMS) to ensure seamless data integration and minimize the risk of human intervention. Through this “process interception + terminal verification” strategy, lifecycle management of impurity risks can be achieved.

5.3. Strengthening personnel training and skill improvement

Personnel training is a core element in improving the quality of pharmaceutical impurity analysis. The professional knowledge and comprehensive abilities of analysts directly affect the accuracy and credibility of test results. Systematic training can help analysts quickly master cutting-edge detection techniques and methods, such as the operational skills and maintenance points of equipment like ultra-high-performance liquid chromatography and mass spectrometry, enabling them to skillfully use advanced tools for work. The training content should also comprehensively cover knowledge about drug impurities, including their sources, properties, and mechanisms affecting drug quality, to reinforce theoretical foundations. Additionally, practical operation training should be intensified to enhance analysts’ problem-solving abilities through extensive hands-on experience. Furthermore, emphasis should be placed on cultivating a rigorous scientific attitude and a strong sense of responsibility, ensuring that drug impurity analysis results are accurate and effective from all dimensions, and providing solid human support for drug quality control.

5.4. Utilizing artificial intelligence and automation technology

Artificial intelligence and automation technology have immense potential in the field of pharmaceutical impurity analysis. Automation technology enables processes such as sample pretreatment, instrument operation, and data collection to be automated, significantly reducing errors caused by manual operations and enhancing analysis efficiency. For instance, automated equipment can accurately perform sample extraction, filtration, and other pretreatment steps, and can also control instruments according to preset programs, ensuring the stability of the analytical process. Artificial intelligence, leveraging machine learning algorithms, deeply mines massive amounts of impurity analysis data to discern potential patterns and trends, aiding in determining the source and properties of impurities. For example, AI combined with spectral analysis and deep learning can perform high-precision authenticity identification of medicinal materials like goji berries and *Cordyceps*, while tracing their origin (such as ginseng mainly produced in Northeast China) [9]. Additionally, AI plays a key role in data quality control, automatically screening abnormal data, further enhancing the accuracy and reliability of data analysis. For instance, through deep learning analysis of drug-target interactions, potential antiviral drugs (such as antiviral components in traditional Chinese medicine compounds) [10] can be quickly screened. The integration of these technologies creates smarter and more efficient technical solutions for drug quality control.

6. Conclusion

Impurity analysis in drug testing plays a crucial role in quality control. It identifies and controls harmful impurities, ensuring drug purity, stability, safety, and effectiveness. Among current impurity analysis techniques and methods, chromatographic and mass spectrometry techniques have their applications, but challenges such as improving analytical sensitivity and accuracy, addressing complexities in impurity analysis of complex samples, and method reproducibility still exist. In the future, drug impurity analysis needs continuous optimization. On one hand, advanced technologies like ultra-high-performance liquid chromatography and chromatography-mass spectrometry should be introduced to enhance analytical efficiency and accuracy. On the other hand, standardized work processes should be established, personnel training should be strengthened, and artificial intelligence and automation technology should be utilized to improve analysis quality and efficiency. It is believed that with advancing technology and refined methods, drug impurity analysis will play a greater role in drug quality control, providing stronger support for ensuring public medication safety and promoting the healthy development of the pharmaceutical industry.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Chen H, 2024, Application of High-Performance Liquid Chromatography in Food and Drug Testing. Sino-Foreign Food Industry, (08): 46–48.

[2] Guo H, 2024, Analysis of the Application of Ion Chromatography in Drug Detection. Brand and Standardization, (06): 39–41.

[3] Zhu L, Pan Y, 2024, Pharmaceutical Quality Inspection Technology, Chemical Industry Press, Shanghai.

[4] Zhang L, Huang W, Li W, et al., 2024, Application of Standardized Chemical Analysis Technology in Drug Safety Testing. China Standardization, (20): 158–161.

[5] Tian Y, Tao X, Feng Y, et al., 2024, Quality Control of Genotoxic Impurities in Chemical Drugs. Chinese Journal of Antibiotics, 49(01): 13–25.

[6] Shao W, Chen Y, Yuan H, et al., 2023, Two-Dimensional Ultra-High Performance Liquid Chromatography-Quadrupole/Time-of-Flight Mass Spectrometry for the Analysis of Teicoplanin Impurities. Chromatography, 41(02): 195–204.

[7] Hao G, 2019, Progress in the Application of Supercritical Fluid Chromatography in Drug Component Analysis. Tianjin Pharmacy, 31(05): 75–78.

[8] Wang S, Zhao X, Liu G, et al., 2024, Research Progress on the Supervision and Inspection Technology of Nitrosamine Impurities in Drugs. Drug Evaluation Research, 47(07): 1631–1637.

[9] Wang C, Zhang X, Gu Z, et al., 2024, Discussion on the Application of Artificial Intelligence in the Detection of Chinese Herbal Medicines and Decoction Pieces. Engineering Science, 26(02): 245–254.

[10] Shan S, Wen B, Qiao T, et al., 2024, Application and Problem Analysis of Artificial Intelligence in Drug Reuse for the Treatment of COVID-19. Chinese Journal of Pharmacology and Toxicology, 38(04): 294–303.