Research Progress in Pre-treatment and Detection Technologies for Veterinary Drug Residues

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Abstract In recent years, the problem of veterinary drug residues in animal-derived foods has attracted worldwide attention. Developing rapid, simple, highly sensitive, and high-throughput veterinary drug residue detection technologies has become an urgent need. This paper provides a comprehensive review of the pretreatment and analytical techniques for veterinary drug residue analysis, comparing the detection principles, operational procedures, and respective advantages and disadvantages of various detection technologies. It further explores the future development directions of veterinary drug residue detection technologies.

Key words Veterinary drugs; Pretreatment; Detection technology

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Pre-treatment Techniques for Veterinary Drug Residues

Sample pre-treatment is the process of extracting and purifying target compounds from samples to be tested. Based on the physical and chemical properties of sample matrices and target analytes, samples undergo preparation, extraction or enzymatic hydrolysis, purification, and concentration. Due to the complexity of samples, when they contain abundant fats, proteins, or sugars, the matrix effect (ME) can significantly increase, which may interfere with target detection and contaminate the mass spectrometer, particularly in trace analysis[1].

According to the literature, during the analysis of a batch of samples, pre-treatment accounts for two-thirds of the total time, and approximately 60% of failures are attributed to this step. Effective pre-treatment not only reduces the impact of matrix and instrument contamination, but is also crucial for minimizing instrument maintenance and enhancing the sensitivity, accuracy, and precision of analytes. Therefore, selecting and designing a well-considered sample processing procedure is of significant importance for ensuring the accuracy of experimental results.

Most current sample pre-treatment methods are designed for analyzing one or multiple types of residues. During the detection process, the high consumption of pre-treatment reagents can easily lead to pollution of water sources, air, soil, and other environmental components. Thus, while ensuring food safety through testing, there is a risk of causing secondary environmental pollution. Due to the frequent occurrence of drug abuse, the scope of analytical targets, sample volumes and the difficulty of detection have all increased significantly[2]. Traditional detection methods are no longer sufficient to meet current demands, and new pre-treatment approaches are constantly emerging. The following are several main pre-treatment methods currently commonly used for determining veterinary drug residues.

Liquid-liquid extraction (LLE)

Liquid-liquid extraction is one of the classical extraction and purification methods. Its principle involves separating target residues with different partition coefficients between two immiscible solvents, and it is widely used for the extraction of similar related substances[3]. This method is matrix-driven and offers relatively weak purification effects. It can be used for quantitative screening of some veterinary drug residues and is often applied for semi-quantitative screening of residues from several veterinary drug products. Extraction solvents should be selected to minimize harm to human health and environmental pollution. Acetonitrile, dimethyl sulfoxide, ethyl acetate, methanol, and cyclohexane are commonly used. The advantages of this method include not requiring specialized equipment, and being inexpensive and easy to operate. However, it involves high reagent consumption, poses significant health risks to operators and is relatively time-consuming. It also entails cumbersome procedures. Additionally, emulsification can occur during the operation[4]. Ultrasonic-assisted liquid-liquid extraction is an environmentally friendly technique. It involves emitting ultrasound waves at liquid or liquid-solid interfaces, generating numerous bubbles that then collapse instantaneously, creating shockwaves. When the bubbles collapse, the target analytes are transferred into the extraction solvent.

Solid-phase extraction (SPE)

Solid-phase extraction (SPE) separates measurable substances from impurities through selective adsorption and selective elution. This means that target substances in a liquid sample are adsorbed, eluted, and desorbed via heating, thereby achieving rapid separation and purification[5]. An appropriate solid-phase

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extraction cartridge must be selected based on the properties of the analyte to achieve optimal separation and purification. Solid-phase extraction is a four-step process involving activation, loading, washing, and elution, and it is a method frequently used today. It is simple, rapid, and efficient, consumes minimal reagents, and can be easily integrated with large-scale equipment. Commonly used SPE cartridges include hydrophilic-lipophilic balanced (HLB) columns, mixed-mode strong anion exchange (MAX) columns, and mixed-mode strong cation exchange (MCX) columns.

The novel reversed-phase solid-phase extraction cartridge eliminates the need for activation and equilibration steps. After extraction, the sample solution can be passed directly through the cartridge. Its unique frit design and packing technology allow for rapid passing relying on gravity, saving reagents and improving efficiency. This method is environmentally friendly. It achieves up to 95% removal of matrix interferences such as proteins, reduces matrix effects, and enhances analytical accuracy and sensitivity.

QuEChERS method

The QuEChERS method was developed in the early 21st century, initially for detecting pesticide residues in agricultural products. Since then, it has been applied to the analysis of veterinary drug residues because it is rapid, simple, and reliable for the simultaneous detection of multiple compounds. The principle involves adsorbing, separating, and purifying the impurities by the interaction between absorbent fillers and impurities in the matrix[6]. With ongoing research, this technique is increasingly being applied in animal feed, processed foods, pharmaceuticals, and environmental fields. The process is straightforward, requiring only homogenization, the addition of water and detergents, and parameter setting on an automated QuEChERS machine, followed by direct injection of the supernatant into the machine. It eliminates the drawbacks of manual enrichment, elution, concentration, and evaporation of target compounds. This method saves a significant amount of organic chemicals, improves efficiency, and reduces economic costs.

Accelerated solvent extraction (ASE)

Accelerated solvent extraction is a pre-treatment method that uses organic solvents to automatically extract solid or semi-solid samples under high temperature and pressure. This method enhances the solubility and diffusion efficiency of target analytes. High temperature increases analyte solubility and reduces matrix effects, while high pressure keeps the solvent in a liquid state at elevated temperatures, preventing the evaporation of volatile components and shortening extraction time[7]. However, the high cost of equipment required for this technique has limited its widespread application in the analysis of veterinary drug residues in animal-derived foods.

Matrix solid-phase dispersion (MSPD)

Matrix solid-phase dispersion is a rapid sample pre-treatment technique proposed in the 1980s. It allows for the extraction and purification of samples to be completed in a single step. The uniqueness of this technique lies in its simultaneous homogenization, extraction, and purification of the sample, eliminating the need for transfer and solid-phase extraction cartridge steps. This reduces the loss of target compounds and saves time costs in experiments. Due to limitations in grinding particles and packing processes, standardizing this method remains challenging[8]. In recent years, some researchers have summarized the application of matrix solid-phase dispersion for detecting veterinary drug residues in animal-derived foods[9].

Detection Techniques for Veterinary Drug Residues

Veterinary drugs in animal-derived foods can accumulate and enrich in the human body through the food chain, posing risks to human health. Attention to the detection of veterinary drug residues has been increasing both at home and abroad, with particular focus on the detection of steroid hormones, nitroimidazoles, β-agonists, sedatives, and similar drugs in animal-derived foods.

With societal development and advances in science and technology, detection methods for veterinary drug residues in animal-derived foods are also evolving toward rapid efficiency, high sensitivity, and environmental friendliness. Mass spectrometry screening technology is an analytical approach that separates and detects compounds based on their mass-to-charge ratio (m/z). By detecting the mass-to-charge ratio of compounds, accurate qualitative and quantitative analyses are achieved. High-resolution mass spectrometry (HRMS) offers exceptional resolution and ultra-high precision mass analyzers for qualitative screening, effectively compensating for the limitations of triple quadrupole mass spectrometry, which relies on reference standards and has a narrower scope[10]. Liquid chromatography-mass spectrometry (LC-MS) combines the high-efficiency separation of chromatography with the precise qualitative capability of mass spectrometry. It offers advantages such as high sensitivity, strong selectivity, and a broad application range, making it the "gold standard" for analyzing trace compounds[11].

High-resolution mass spectrometry (HRMS) based on liquid chromatography (LC) has become an essential tool for detecting veterinary drug residues. Ecological and efficient extraction methods represent the developmental direction for pre-treatment approaches. Advances in chromatography and column packing materials, improvements in resolution, and the optimization of application software will further drive the application of this technology in the determination of veterinary drug residues.

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) accomplishes continuous separation between two phases primarily by leveraging differences in the boiling point, polarity, adsorption, and other physical properties of samples. Based on differences in the fragmentation patterns of substances, the ion source facilitates the passage of charged ions through the detector. These ions are then processed by an electrical signal amplifier, and the computer system calculates the results. This method is suitable for volatile,

small-molecular-weight, and thermally stable compounds. It is easy to operate, time-efficient, and environmentally friendly. GC-MS is widely used in environmental and pesticide analysis due to its robust detection, and high sensitivity and accuracy. However, most veterinary drugs have high polarity and boiling points, requiring extensive derivatization processes for detection by GC. Since the 1980s, the development of high-performance liquid chromatography (HPLC) has surpassed that of GC, with drugs such as sulfonamides and chloramphenicol being detected using HPLC[12].

High-performance liquid chromatography (HPLC)

The fundamental principle of high-performance liquid chromatography (HPLC) is that solvents of different polarities are separated or mixed with the mobile phase in the stationary phase based on polarity differences, after which they are detected by the detector and converted into electrical signals. HPLC is relatively inexpensive and easy to operate, making it widely used in pharmaceuticals, chemistry, agriculture, the food industry, and research. However, HPLC lacks a universal detector that meets the requirements for veterinary drug residue analysis. Commonly used detectors include ultraviolet detectors, fluorescence detectors, and electrochemical detectors. Over the past decade, the most significant development in HPLC has been the diode array detector.

Liquid chromatography-mass spectrometry (LC-MS)

Research on liquid chromatography-mass spectrometry (LC-MS) began in the late 1960s. LC-MS largely addresses the limitations of GC-MS, as it can analyze samples with low volatility, water solubility, and thermal instability. It allows for the separation of more polar, high-molecular-weight, non-volatile, and thermally unstable samples. These compounds constitute up to 80% of total organic matter. Combined with the powerful qualitative capability of mass spectrometry for structural analysis by extracting compound fragment ions, LC-MS has broad applications in the field of organic analysis[13].

Types of mass spectrometers

Given the extensive application of LC-MS, the selection of mass spectrometers has become a critical point. Triple quadrupole mass spectrometers (TSQ-MS) offer strong quantitative and qualitative capabilities, with advantages such as high specificity and sensitivity. They are already widely used for determining trace veterinary drug residues in animal-derived foods[14]. Sector field mass spectrometers (Sector-MS) change the magnetic field strength and use secondary electron avalanche voltage detectors for acceleration. Ions, under the influence of Lorentz force, can obtain accurate mass numbers, achieving high resolution. Although they possess exceptional quantitative capabilities, good stability, high sensitivity, and high resolution, these instruments are bulky, structurally complex, difficult to operate, and relatively slow in analysis speed[15]. Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) uses a magnetic field to induce ion cyclotron motion, and creates resonance conditions through rapid sweeping and radiofrequency voltage, and following computer-based fast Fourier transformation, mass spectra with ultra-high mass accuracy can be obtained. This achieves high resolution and is unaffected by signal detection time and resolution limitations. It can simultaneously measure the mass-to-charge ratio and abundance ratio of all ions. However, the instrument has the largest physical footprint among mass spectrometers, requires a superconducting magnet and a high-vacuum system, and places high demands on operators[16]. The bulky size and operational complexity of Sector-MS and FTICR-MS instruments limit their widespread use, and they are primarily employed for analyzing complex samples.

Prospects

The increasing public concern over food safety and the growing stringency of international trade requirements for food safety impose higher demands on veterinary drug residue analysis technology, while also presenting opportunities for its development. The rapid advancement of modern analytical technologies provides fundamental technical assurance for the establishment and improvement of veterinary drug residue detection methods. The emergence of new analytical approaches and the complementary application of multiple techniques are progressively addressing various challenges in veterinary drug residue analysis, such as complex matrices, the wide variety of veterinary drugs, and low concentration levels. Currently, various countries are continuously strengthening the monitoring and management of veterinary drug residues, imposing increasingly higher demands on data accuracy and expanding both the scope of monitoring and sample sizes. This necessitates further advancements in veterinary drug residue detection technology to meet these evolving needs. The development direction of veterinary drug residue detection technology can be summarized as follows: (1) higher sensitivity, and lower detection limits and quantification limits; (2) high throughput (analyzing more samples per unit of time); (3) multi-residue analysis (simultaneous determination of multiple veterinary drug residues); (4) higher selectivity, specificity, and anti-interference capability; (5) increased automation and miniaturization of analytical instruments, and integration of pre-treatment and detection; (6) advancement of more hyphenated techniques; and (7) pollution reduction and achieving environmental friendliness. Evidently, veterinary drug residue analysis technology still possesses significant room for development. More scholars are needed to join the research in this field, and continuously dedicate efforts to the development and application of analytical methods across the three levels of veterinary drug residue screening, quantification, and confirmation.

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