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Overview of Veterinary Drug Residues in Animal-Derived Foods and Detection Technologies

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Abstract: Veterinary drug residues pose a global challenge to the safety of animal-derived foods, presenting significant potential threats and hazards to human health. Based on this, this paper systematically reviews optimization strategies for enhancing analytical sensitivity and efficiency in three veterinary drug residue pretreatment techniques: liquid-liquid extraction, solid-phase extraction, and QuEChERS. It elaborates on detection technologies including microbial suppression, immunoassays, high-performance liquid chromatography, gas chromatography-mass spectrometry, liquid chromatography-tandem mass spectrometry (LC-MS/MS), surface-enhanced raman spectroscopy (SERS), electrochemical sensor technology, nucleic acid aptamer sensors, AI-assisted data analysis, and biomimetic recognition materials. This aims to strengthen veterinary drug residue regulation, standardize the healthy development of animal husbandry, increase investment in detection technology R&D, achieve international alignment of detection techniques, and provide technical support for ensuring the safety of food derived from animals.

Keyword: Animal-derived food; Veterinary drug residues; Pretreatment methods; Detection techniques

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1. Preamble

With the continuous development of social economy and the significant improvement of people's living standards, food safety issues are increasingly being highly concerned by the whole society, in which veterinary drug residues have become one of the focus issues[1]. Veterinary drug residues[2] refer to three types of substances that may be present in the edible portion of foods of animal origin (e.g., meat, eggs, milk, etc.): the proto-compounds (parent compounds) of veterinary drugs, the products of their metabolic transformations in the animal body, and the impurities associated with the process of production or use of veterinary drugs. Moreover, as an important source of high-quality proteins, essential amino acids, and a variety of micronutrients in the human diet[3], food of animal origin has an irreplaceable role in daily life.

However, in recent years, about 30% of the cases of unqualified food of animal origin notified by China originated from the pesticide and veterinary drug residue program does not meet the standard[4]. Prolonged intake of food of animal origin with excessive residues of veterinary drugs may cause potential hazards to human health in various aspects, including but not limited to the emergence of bacterial resistance, chronic toxicity, allergic reactions, ecological risks, etc., and may even cause certain impacts on the ecological environment[5].

Therefore, in order to prevent and control the contamination of food of animal origin, it is of great significance to strengthen the research on veterinary drug residue detection. Veterinary drug residue detection technology is a professional technology that analyzes the level of drug residues in livestock and poultry products to determine whether they meet the safety limits[6]. The first and foremost prerequisite for accurate detection is efficient sample pre-treatment. Due to the complexity of the matrix of animal tissues, milk and other samples, which are rich in proteins, fats and other interferences, it is necessary to extract, purify and enrich the sample before obtaining pure samples that can be accurately analyzed by the instrument. In livestock production, veterinary drugs are indispensable for disease prevention and control, but if the use of drugs is not standardized or the rest period is not strictly enforced, it is easy to lead to excessive drug residues in the products, which constitutes a potential threat to human health. Therefore, the establishment and implementation of a rigorous sample pretreatment process, followed by timely and accurate testing, is a key measure for prevention and control.

2. Pre-treatment techniques for veterinary drug residue samples

Sample pretreatment is a key step in veterinary drug residue testing to efficiently extract, purify and enrich target compounds from complex animal tissues or body fluids.

2.1. Liquid-liquid extraction

Liquid-liquid extraction(LLE) is a classical sample pretreatment technique, the core principle of which is to utilize the difference in partition coefficients of the substances to be measured in two immiscible solvents[7], enables the isolation, purification and enrichment of analytes for target veterinary drug residues from complex sample matrices. However, the limitations of traditional LLE are also very significant: it usually consumes a large amount of organic solvent, the operation steps are cumbersome and time-consuming, it is difficult to realize automation and high-throughput processing, and when dealing with complex biological samples rich in proteins or lipids, it is prone to emulsification phenomenon, which affects the efficiency of the separation and the reproducibility of the results. Sun et al[8] successfully developed a method based on salting-out-assisted liquid-liquid extraction technique combined with high performance liquid chromatography-tandem mass spectrometry HPLC-MS/MS, which, unlike the traditional LLE that uses organic solvents directly for extraction, used Na_2EDTA-Mcllvaine buffer or ACN as the extraction solvent system. After homogenizing the sample, mixed salts of $MgSO_4$ and NaCl were added. The “salting-out effect” produced by dissolving these salts reduces the polarity of the aqueous phase, significantly decreasing the solubility of target veterinary drugs in water. This forces them to distribute more efficiently into the acetonitrile organic phase, thereby enhancing extraction recovery. Consequently, liquid-liquid extraction is often employed as a primary extraction method or combined with modern pretreatment techniques like solid-phase extraction or QuEChERS, which offer higher selectivity and throughput.

2.2. Solid-phase extraction

In veterinary drug residue detection, Solid-phase extraction(SPE) primarily achieves purification, separation, and enrichment by selectively retaining target veterinary drugs in the sample solution using adsorbents, followed by selectively eluting impurities and analytes with solvents[9]. However, traditional solid-phase extraction methods involve manual operations and cumbersome procedures. Sun et al[10] developed a direct-through solid-phase extraction method. Unlike conventional SPE, which requires multiple steps such as activation, sample loading, rinsing, and elution, the design of the direct-through SPE column allows the sample extract to pass through directly. Under positive or negative pressure, the solution flows through the purification packing material within the column. The packing selectively adsorbs interfering substances such as fats and proteins from the sample matrix, while the target veterinary drug molecules elute with the solution and are collected. This method, coupled with ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), has been successfully applied to detect 17 veterinary drug residues in animal-derived foods, with sensitivity fully meeting international maximum residue limits. This technology is particularly well-suited for high-

throughput testing laboratories, significantly enhancing operational efficiency.

2.3. QuEChERS

QuEChERS is a highly efficient and versatile sample preparation technique. Its core purification mechanism draws upon the fundamental principles of solid-phase extraction, leveraging the selective interaction between specific adsorbents and interfering impurities within the sample matrix to achieve effective purification of target analytes[11]. Zhang et al.[12] optimized and innovated the traditional QuEChERS method. This study employed a mixture of glacial acetic acid-acetonitrile-water (1:84:15,V/V) as the extraction solvent and incorporated sodium ethylenediaminetetraacetate Na_2EDTA. This design is ingeniously conceived: the acidic environment enhances the extraction efficiency of quinolone and tetracycline antibiotics, while Na_2EDTA effectively chelates metal ions potentially present in visceral tissues, preventing their binding with tetracycline drugs that could reduce recovery rates. The purification step employs DSC-18 adsorbent with optimized dosage, ensuring purification efficacy while minimizing adsorption of target drugs.

Purified samples undergo dual confirmation via multi-reaction detection mode, achieving precise qualitative and quantitative analysis. Through continuous optimization, QuEChERS technology now effectively addresses challenges posed by complex matrices such as high-fat, high-protein animal viscera. Seamlessly integrated with modern high-precision mass spectrometry, it has evolved into a mature, efficient, and reliable cutting-edge solution for veterinary drug residue detection. Through continuous optimization, the QuEChERS technique has evolved into a mature, efficient, and reliable cutting-edge solution for veterinary drug residue detection. It effectively addresses challenges posed by complex matrices such as high-fat, high-protein animal offal and seamlessly integrates with modern high-precision mass spectrometry technologies.

2. Methods for detecting veterinary drug residues

2.1. Microbial inhibition method

Microbial inhibition method(MIA)[13] is a traditional method for rapid screening of veterinary drug residues in animal-derived foods based on the physiological inhibitory effects of antibiotics on specific susceptible microorganisms. The core principle of this method is that if antibiotic residues are present in the sample, they will inhibit the growth of susceptible bacterial strains. Gao et al.[14] improved the indicator bacteria and detection system by innovatively selecting Escherichia coli as the indicator organism. This strain demonstrated good sensitivity to both quinolone and aminoglycoside antibiotics, thereby expanding the detection range. They also incorporated the pH indicator bromocresol purple into the culture medium. When indicator bacteria undergo normal growth and metabolism, they produce acid, lowering the pH of the culture medium and causing the indicator color to change from purple to yellow. If antibiotics are present in the sample, bacterial growth is inhibited, metabolism ceases, and the medium color remains unchanged (purple). This strategy of translating growth inhibition into a visual color change enables more objective and convenient result interpretation. Additionally, a 96-well microplate is employed as the reaction carrier, enabling simultaneous processing of dozens of samples for high-throughput detection. Its advantages of speed, cost-effectiveness, and suitability for large-scale initial screening are particularly prominent, making it ideal for rapid risk monitoring at sites such as farms and slaughterhouses.

2.2. Immunoassay

Immunoassays are based on the specific recognition and binding reaction between antigens and antibodies. By detecting the signal changes generated by this reaction, they enable the qualitative or quantitative analysis of specific veterinary drugs or their metabolites in samples[15]. Immunoassay methods primarily include ELISA[16], chemiluminescent immunoassay, and fluorescent immunoassay. Among these, the ELISA technique has a mature and well-established system. In a study by Han et al.[17] novel bifunctional mesoporous silica nanocomposites were used to replace conventional enzyme-labeled secondary antibodies. This material possesses an extremely high specific surface area, enabling simultaneous loading of

large quantities of signal molecules (such as fluorescent dyes or enzymes) and recognition elements. During detection, a single immune recognition event can trigger hundreds of signal molecules to generate a response, thereby significantly amplifying the detection signal. Research indicates that this method achieves 16.7 times higher sensitivity than traditional ELISA for detecting multiple veterinary drug residues (e.g., avermectin, chloramphenicol), while demonstrating excellent reproducibility and accuracy. It is highly suitable for high-throughput screening of trace and multiple residues in complex food matrices.

2.3. Chromatography

Chromatography is currently the core technical method for detecting veterinary drug residues in animal-derived foods, particularly excelling in the precise qualitative and quantitative analysis of multiple drugs within complex sample matrices.

2.3.1. High-performance liquid chromatography

The core principle of High-performance liquid chromatography(HPLC)[18] for detecting veterinary drug residues relies on separating components based on their differing distribution behaviors between the stationary phase and mobile phase within the sample. Due to variations in physicochemical properties such as polarity and size among different veterinary drugs, they migrate at distinct rates through the chromatographic column, enabling separation. Subsequently, the separated components enter the detector for qualitative and quantitative analysis[19]. Currently, the most advanced technology in this field is ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry. Through its exceptional mass accuracy and resolution, this technique enables precise screening and confirmation of trace amounts of multiple categories of veterinary drug residues in complex samples. A study by Xia et al.[20] successfully established a method using UPLC-Q-TOF/MS for the simultaneous detection of over 100 veterinary drug residues in chicken and pork, covering multiple categories of compounds including antibiotics, synthetic antimicrobials, and antiparasitic drugs. The method achieved detection limits at or below the μg/kg level for all target veterinary drugs. Spiked recovery rates in chicken and pork matrices remained stable between 70% and 120%, fully meeting the most stringent international residue limits. UPLC-HRMS technology maximizes the separation capabilities of chromatography and the precise identification capabilities of mass spectrometry. Its high throughput, high sensitivity, and robust non-targeted screening capabilities make it a powerful tool for discovering unknown risk residues. With decreasing instrument costs and increasingly intelligent data analysis software, this technology is moving beyond elite laboratories toward broader applications, providing strong technical support for ensuring the safety of livestock and poultry products.

2.3.2. Gas chromatography-mass spectrometry

GC-MS combines the high-efficiency separation capability of gas chromatography with the precise qualitative analysis capability of mass spectrometry, making it a powerful technique for detecting veterinary drug residues. Its core principle relies on separating compounds based on differences in boiling points, polarity, and adsorption properties[21], followed by individual identification and quantification of their components. It is primarily applied to small-molecule, volatile, thermally stable, and vaporizable compounds. Wang et al.[22] developed a gas chromatography-mass spectrometry method for the simultaneous determination of sulfamethoxazole, florfenicol, and its metabolite florfenicolamine residues in chicken and pig muscle, fat, liver, and kidney tissues. Addressing the challenge of flumefadone's high polarity, which hinders direct GC-MS analysis, the study employed BSTFA for derivatization. This step converts target compounds into volatile, thermally stable derivatives, significantly improving their separation behavior on the chromatographic column and detection sensitivity. This is crucial for achieving simultaneous detection of multiple residues. This method employs a negative chemical ionization source. Compared to conventional electron impact sources, the NCI source exhibits higher sensitivity toward compounds containing strongly electronegative atoms (such as halogens), including amide-based drugs. It significantly lowers detection limits and is suitable for trace residue analysis. With its high sensitivity and excellent accuracy, this method provides a robust technical tool for ensuring the safety of livestock and poultry products.

2.3.3. Liquid chromatography-tandem mass spectrometry

The core principle of LC-MS/MS detection for veterinary drug residues lies in its ability to first separate the components within a mixture using high-performance liquid chromatography, followed by precise identification and quantification of each component through the accuracy of mass spectrometry. This method is particularly suitable for confirmatory analysis of veterinary drugs in trace amounts, multiple residues, and complex matrices. For example, Fedeniuk et al.[23] established a method using LC-MS/MS for the simultaneous detection of four amide-type veterinary drug residues (including chloramphenicol and florfenicol) in bovine, equine, and porcine liver. This method demonstrated excellent performance: Detection limits as low as 0.1–1.0 μg/kg, average recovery rates of 50%–90%, and direct detection of conjugated metabolites without enzymatic hydrolysis. This demonstrates the capability of liquid chromatography-mass spectrometry for highly sensitive and confirmatory detection of trace, multi-category veterinary drug residues in complex matrices of animal-derived foods.

2.4. Rapid detection technology

2.4.1. Surface-enhanced raman spectroscopy

SERS is an exceptionally sensitive detection technique that amplifies the Raman signal of target molecules by millions or even billions of times through specialized metallic nanostructures[24], enabling the precise identification of minute residues of veterinary drugs. Wang et al.[24] developed a label-free SERS detection platform integrating dual signal enhancement with machine learning algorithms. Employing a dual strategy of calcium borohydride activation and calcium ion-deuterium oxide guidance, the platform efficiently captures and concentrates target drug molecules within the highly active regions of nanoparticles, yielding high-quality SERS spectra. y applying multiple machine learning algorithms such as PCA-LDA (Principal Component Analysis-Linear Discriminant Analysis) and decision tree modeling, the system intelligently parses complex spectra to accurately distinguish and identify various veterinary drugs and mixed samples. The quantitative analysis results in meat samples showed high consistency with HPLC measurements, demonstrating its reliability and high sensitivity for detection in complex food matrices. Beyond the aforementioned machine learning strategies, another cutting-edge technological example is SERS detection technology based on flexible substrates and novel nanomaterial composite structures. The study utilizes readily available polyurethane yarn as a flexible substrate, functionalized via polydopamine modification. Leveraging the reductive properties of polydopamine, silver ions from silver ammonia solution were reduced in situ on the yarn surface, forming uniformly distributed silver nanoparticles. This process ultimately yielded a PU@PDA@AgNPs composite flexible SERS substrate [25]. Through the development of flexible, low-cost, and easily fabricated substrate materials, SERS technology is transitioning from the laboratory to the field, enabling rapid initial screening in scenarios such as livestock farms, slaughter processing lines, or market supervision.

2.4.2. Electrochemical sensor technology

Electrochemical sensors for detecting veterinary drug residues represent a cutting-edge technology. By leveraging the specific binding between biosensors and target veterinary drugs, this approach converts the unique interaction between biosensors and drugs into electrical signals[26], enabling rapid and highly sensitive detection of trace residues. Guo et al.[27] employed a DNA aptamer capable of specifically recognizing sulfamethoxazole. Using mesoporous silica nanospheres as carriers, they preloaded the electrochemical signal molecule methylene blue within their pores. In the absence of the target compound, the aptamer acts like a lid, adsorbing onto the mesoporous material surface via electrostatic interactions and trapping methylene blue within the pores, resulting in a weak electrical signal. When sulfadimethoxine is present in the sample, the aptamer preferentially binds to sulfadimethoxine, causing a conformational change and detachment from the mesoporous surface. The “lid” opens, releasing methylene blue into the solution and generating a significantly enhanced electrochemical signal. The signal enhancement is directly proportional to the concentration of sulfadimethoxine, enabling quantitative detection. This fully demonstrates the unique advantages of electrochemical sensor technology: rapid response, high sensitivity, and suitability for on-site screening. Compared to traditional chromatography-mass spectrometry methods, it eliminates the need for complex, expensive instruments and cumbersome pretreatment, offering a highly promising and

efficient solution for routine monitoring of veterinary drug residues in livestock and poultry products.

2.5. Emerging technologies

Emerging technologies are revolutionizing veterinary drug residue detection, with nucleic acid aptamer sensors, biomimetic recognition materials, and AI-assisted data analysis standing out as particularly prominent.

2.5.1. Nucleic acid adaptor sensor

The core principle of nucleic acid aptamer sensors for detecting veterinary drug residues involves obtaining single-stranded DNA/RNA fragments (aptamers) with high specificity and affinity for binding target substances (such as specific veterinary drugs) through in vitro screening. These aptamers serve as recognition elements that undergo conformational changes upon binding to the target substance[28]. A study by Sun et al.[29] developed a dual-mode nucleic acid aptamer sensor driven by DNA walkers and catalyzed by G-quadruplex DNAzymes for highly sensitive detection of cephalosporin antibiotics in milk. Its innovation lies in employing real milk matrix-assisted SELEX technology to screen for high-affinity aptamers, and designing a target-triggered DNA walker molecular machine capable of "walking" on magnetic bead surfaces. This achieves initial amplification through stepwise cleavage generating cumulative fluorescence signals, followed by G-quadruplex/ chlorheme complex-catalyzed colorimetric reaction to generate a colorimetric signal, forming a dual-mode fluorescence-colorimetric output. Its dual-signal self-calibration mechanism significantly enhances detection accuracy and interference resistance in complex matrices, representing a crucial direction for the development of aptamer sensors toward intelligent, practical point-of-care testing.

2.5.2. Bionic recognition materials

The core of bionic recognition materials for detecting veterinary drug residues lies in the artificial synthesis of high-molecular-weight polymers with predetermined recognition capabilities for specific veterinary drug molecules. Through spatial structural complementarity and chemical interactions, these materials achieve specific binding to target molecules. The core of preparing biomimetic recognition materials lies in molecular imprinting technology[30]. Jian et al.[31] successfully developed a paper-based borate affinity metal-organic framework/molecularly imprinted polymer (FZS-BA@MIP) microfluidic chip for rapid, highly sensitive detection of kanamycin residues in livestock and poultry products. The research team employed a borate-affinity surface imprinting strategy to prepare a molecularly imprinted polymer (MIP) as the recognition layer. Using the target veterinary drug kanamycin (an aminoglycoside antibiotic) as a template, they polymerized around it to form a macromolecular polymer. After eluting the template molecules, the polymer retained cavities perfectly complementary to kanamycin in both shape and chemical structure.

This MIP exhibits antibody-like high specificity in recognizing and capturing kanamycin in samples, yet offers superior stability and cost-effectiveness compared to natural antibodies. It withstands harsh environments and enables reuse. The technology incorporates zeolite imidazolate framework material (ZIF-8), a metal-organic framework (MOF). ZIF-8 possesses a vast specific surface area and well-defined pore channels, enabling it to concentrate kanamycin molecules in samples like a sponge, thereby amplifying the signal. Ultimately, integrating MIP and MOF onto a paper-based microfluidic chip enables automatic enrichment, recognition, and reaction of sample solutions through capillary action, achieving visual detection within 30 minutes. This provides a powerful innovative approach for rapid, highly sensitive, and low-cost on-site screening of trace veterinary drug residues in complex livestock and poultry products.

2.5.3. AI-assisted data analysis

Artificial intelligence is revolutionizing data analysis in veterinary drug residue detection. At its core, it leverages algorithms such as machine learning and computer vision to transform processes traditionally reliant on human expertise into automated, intelligent decision-making systems. This enables breakthrough improvements in speed, accuracy, and scale[32]. Dong et al.[33] scanned 300 lamb meat samples with varying concentrations of ofloxacin residues using

a near-infrared hyperspectral imaging system. This system simultaneously captures spatial image information and continuous spectral data from the samples, obtaining the spectral profile of each pixel point in the near-infrared band. A deep learning model—the convolutional neural network-stacked sparse autoencoder—was employed to process the massive hyperspectral dataset. This model automatically learned and extracted deep spectral features closely correlated with ofloxacin residues, effectively reducing dimensionality and removing noise. Based on the extracted features, the SHAP method was applied to analyze the contribution of different spectral features to the prediction results within the model. Mapping the results generated by the predictive model back to the original image space of the samples provides an intuitive visualization of the distribution and concentration variations of ofloxacin within the lamb samples. This approach eliminates the need for complex chemical pretreatment of samples, enabling rapid, non-destructive screening that facilitates online quality monitoring. Furthermore, the integration of explainable AI technology renders the analytical process comprehensible and trustworthy, which is crucial for its practical implementation.

3. Conclusion

The veterinary drug residue detection system for animal-derived foods has evolved from traditional microbial inhibition and immunoassay methods to a confirmation platform centered on chromatography-mass spectrometry technology, integrating rapid screening with laboratory confirmation. It continues to advance toward emerging technologies characterized by speed, intelligence, and high throughput. This review indicates that current detection technologies exhibit multi-tiered, complementary characteristics: optimized sample preparation techniques significantly enhance analytical sensitivity and efficiency; chromatography-mass spectrometry, as the gold standard, remains irreplaceable for precise quantification of multiple residues in complex matrices; while rapid detection technologies and emerging identification materials markedly improve the convenience and specificity of on-site screening. Notably, AI-assisted data analysis is permeating the entire chain—from sample preparation optimization to intelligent result interpretation—signaling a shift from manual expertise to data-driven decision-making. However, challenges persist, including matrix interference, insufficient standardization, and cost-benefit balancing, which require multi-technique integration and process optimization to overcome.

Disclosure statement

The author declares no conflict of interest.

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