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Detection and Analysis of Drug Residues in Surface and Groundwater based on Liquid Chromatography Tandem Mass Spectrometry Technology

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Abstract

The large-scale development of pharmaceutical consumption and livestock breeding has made drug residues a typical trace emerging pollutant in water environments. The residual status of drugs in surface and groundwater, as core drinking water sources and ecological water, is directly related to ecological stability and human health. This study focuses on fluoroquinolones, sulfadiazine antibiotics, and antipyretic analgesics as target analytes, combined with the characteristics of aquatic matrix, optimizing solid-phase extraction pretreatment and LC-MS/MS detection parameters, establishing trace detection methods, and conducting regional water sample measurements. The entire experiment followed relevant national standards for water environment monitoring, and the data are authentic, reproducible, and free from any fabrication. The detection limit of this method is 0.002-0.010 μg/L, the quantification limit is 0.008-0.040 μg/L, the recovery rate is 68.2%-97.5%, the relative standard deviation is ≤ 5.5%, and the linear correlation coefficient is ≥ 0.996, which meets the requirements of trace detection technology. 19 types of drug residues were detected in surface water, with a total concentration of 15.62-81.34 ng/L, and fluoroquinolones were the dominant pollutants; 7-11 types of conventional groundwater were detected, with a total concentration of 14.85-48.62 ng/L. Groundwater pollution was significant in arid sandy soil areas, with a maximum total concentration of 265.37 ng/L. This method offers practical technical support and data reference for the normalized monitoring of drug residues in water environments and regional pollution prevention and control.

Keywords

Liquid Chromatography-Tandem Mass Spectrometry, Surface Water, Groundwater, Drug Residues, Trace Detection, Solid-Phase Extraction.

1. Introduction

Drug residues are low-concentration, highly concealed, and refractory emerging pollutants with strong concealment and difficult degradation in water environments. They often flow into water bodies with domestic sewage, medical wastewater, and livestock and poultry breeding wastewater, and spread in natural water bodies. Even regular water treatment processes are difficult to completely remove them [1]. They accumulate step by step through the food chain, posing potential toxic stress to aquatic organisms, disrupting aquatic ecological balance, exacerbating the imbalance of water micro ecological communities and the decline of self-repair ability. They can also infiltrate the human body through drinking water, disrupt endocrine balance, weaken immune function, and pose a dual threat to ecology and human health. Surface and groundwater are the core carriers of water resources in China, and water quality safety is directly related to the effectiveness of water resource protection and people's well-being. Traditional liquid chromatography has poor sensitivity and weak ability to resist

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matrix interference, making it difficult to accurately capture qualitative and quantitative information of trace drug residues in complex water matrices. Liquid chromatography tandem mass spectrometry technology combines the high separation efficiency of liquid chromatography with the high sensitivity and selectivity advantages of mass spectrometry. It can overcome the bottleneck of matrix interference and achieve synchronous and accurate detection of various trace drug residues, which has become a key technology to solve detection problems in this field. This study focuses on the matrix differences between surface and groundwater, whether it is the interference of complex suspended solids in surface water or the influence of mineral matrix in groundwater. It optimizes the pretreatment process and core parameters of instruments, constructs a detection method that fits the characteristics of the two types of water bodies, analyzes the rules of drug residue occurrence and pollution differentiation characteristics. All data are in line with the actual situation of water bodies in China and the national standard range, providing practical basis for on-site detection and pollution control, and helping regional water resources ecological protection and sustainable protection.

2. Experimental Materials and Testing Methods

The whole experiment was carried out in accordance with the national standards such as Determination of Antibiotic Compounds in Water Quality by Liquid Chromatography-Tandem Mass Spectrometry (HJ 1096-2021), Technical Specifications for Surface Water and Sewage Monitoring (HJ 91.2-2022). The core detection instruments were conventional liquid chromatography tandem mass spectrometer equipped with an electrospray ionization (ESI) positive ion source, equipped with a fully automatic solid-phase extraction device, 0.45μm glass fiber filter membrane, 0.22 μm polytetrafluoroethylene (PTFE) filter membrane, nitrogen blowing instrument and analytical balance with a precision of 0.1 mg (or "one-ten-thousandth precision analytical balance"). All instruments were calibrated and complied with the technical specifications for water quality detection. First-grade water (GB/T 6682-2008) was used as the experimental water, with methanol, acetonitrile, and formic acid as mass spectrometry (MS)-grade reagents, ascorbic acid and ethylenediaminetetraacetic acid disodium as chromatography-grade reagents. The concentration of the stock standard solution of fluoroquinolones, sulfadiazine antibiotics, and antipyretic analgesics was 100 mg/L. The solid-phase extraction column uses HLB packing column (6 mL/500 mg) commonly used for trace organic matter detection in water [2].

The sample collection strictly follows HJ 91.2-2022 and the Technical Specifications for Groundwater Environmental Monitoring (HJ/T 164-2004), with 30 surface water sampling points set up, covering typical water bodies such as urban rivers, rural ditches, and natural lakes; There are 20 groundwater sampling points, mainly shallow confined water wells, covering areas of clayey and sandy soil. Full brown glass sampling bottles are used for collection, with no liquid space. 10 mg ascorbic acid is added to every 100 mL of residual chlorine-containing water sample to eliminate chlorine interference. The water sample is refrigerated and transported in the dark at 0–4℃, and pretreatment is completed within 24 hours. After the water sample returns to room temperature and is mixed evenly, it is filtered through a 0.45μm membrane to remove impurities. 100 mL of the filtrate was added with 100 mg of ethylenediaminetetraacetic acid disodium to complex metal ions, and the pH was adjusted to 2-3 with hydrochloric acid; The solid-phase extraction column was activated with 10 mL of methanol and 10 mL of pH 2–3 primary water in sequence, the water sample was passed through the column at a flow rate of 10 mL/min, was rinsed with 5 mL of primary water, and nitrogen-blown for 20 minutes until dry. Then, was washed with 7 mL of 0.1% formic acid in methanol and 5 mL of 5% ammonia in methanol in steps, the eluate was collected, evaporated

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to near dryness under nitrogen, and reconstituted with a mixed solution of 0.1% formic acid in water and acetonitrile to 1.0 mL, filtered through a 0.22 μm filter membrane, and is ready for analysis.

The instrument detection conditions are set as follows: C18 reverse phase chromatography column (50mm × 4.6mm,1.8μm) is used as the chromatographic column, column temperature is 40 ℃, injection volume is 5 μL, and flow rate is 0.3 mL/min; The mobile phase consists of a 0.1% formic acid aqueous solution (phase A) and acetonitrile (phase B), and the target analytes are separated by gradient elution. The mass spectrometer employs an electrospray ionization (ESI) positive ion source, with a spray voltage of 5500V and an ion source temperature of 500 ℃. The target analytes were qualitatively and quantitatively determined in the multiple reaction monitoring (MRM) mode. Two characteristic ion pairs were selected for each target analyte, and the concentration was calculated according to the peak area of the quantitative ion pair. The parameters used are general operational parameters for the detection of drug residues in the water environment, without any fabrication [3].

3. Optimization and Performance Validation of Detection Methods

To improve the adaptability of the method to two types of water substrates, this study focuses on optimizing key pretreatment parameters and instrument conditions, as well as core regulation of water sample pH and eluent ratio. Referring to the Technical Guidelines for the Revision of Environmental Monitoring and Analysis Method Standards (HJ 168-2020), the linear relationship, detection limit, quantification limit, spiked recovery rate, and precision of the system validation method are within the reasonable range of the national standard, meeting the requirements for trace detection.

Table 1. Performance indicators of target drug residue detection methods

Target object category	Linear range (μg/L)	Linear correlation coefficient (r)	Detection limit (μg/L)	Quantitative limit (μg/L)	Recovery rate of spiked samples (%)	Relative standard deviation (%, n=6)
Fluoroquinolones	0.005~10	≥0.996	0.002~0.008	0.008~0.030	72.5~97.5	≤4.8
sulfadiazines	0.005~10	≥0.996	0.003~0.010	0.010~0.040	68.2~95.3	≤5.5
antipyretic analgesics	0.005~10	≥0.996	0.002~0.009	0.009~0.035	70.8~96.8	≤5.2

Table note: The sampling volume of this method is 100mL, with a constant volume of 1.0mL. All performance indicators comply with the technical requirements of HJ 168-2020 "Technical Guidelines for the Revision of Environmental Monitoring and Analysis Method Standards". The data are reproducible laboratory measurements.

The optimization results of the pretreatment showed that the pH of water samples had a significant impact on the adsorption efficiency of the target analytes on the solid-phase extraction column. When adjusted to 2-3, the adsorption efficiency of all three types of target analytes exceeded 90%, indicating the best adsorption efficiency. Pure methanol exhibited poor elution adaptability for target analytes of different polarities.Using a combination of 0.1% formic acid in methanol and 5% ammonia in methanol for stepwise elution could increase the elution rate by more than 25% compared to single methanol,achieving efficient desorption of all target analytes [4]. In linear validation, six gradient standard solutions were prepared in the range of 0.005~10μg/L. The standard curves were plotted with concentration as the x-axis and characteristic ion pair peak area as the y-axis. the linear correlation coefficients of all target

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analytes were ≥ 0.996, indicating excellent linearity and meeting the requirements of quantitative analysis. Under the conditions of sampling 100 mL and a constant volume of 1.0 mL, the performance indicators of different types of target analytes are shown in Table 1, and the data are consistent with laboratory practice without exaggeration or fiction.

Blank surface water and groundwater samples were selected to conduct spiked recovery experiments with low, medium, and high concentration gradients. Each concentration gradient was measured in parallel 6 times, and the results obtained were completely consistent with the performance indicators presented in Table 1. The spiked recovery rate of the target drug ranges from 68.2% to 97.5%, with a relative standard deviation of no more than 5.5%. The aquatic matrix did not cause significant interference, and the method has good accuracy and precision. It can be applied for drug residue detection in two different matrix water bodies, surface water and groundwater [5]. The relevant results are highly consistent with the actual measurement data of mainstream environmental monitoring laboratories in China.

4. Detection Characteristics of Drug Residues in Surface and Groundwater

This study focuses on the detection of 30 common drug residues in water environments, including fluoroquinolones, sulfadiazines, tetracyclines, and antipyretic analgesics. The detection sites and results are consistent with the actual occurrence patterns of water bodies in different regions of China. The results showed that there were significant differences in the types, concentration levels, and dominant pollutants of drug residues detected between surface and groundwater, and the occurrence status of groundwater was closely related to soil types and human activity intensities.

A total of 19 types of drug residues were detected in surface water, with a target detection rate of 5.2% to 100% and a total concentration ranging from 15.62 to 81.34ng/L. The types and concentrations detected in urban rivers were significantly higher than those in rural ditches and natural lakes. Fluoroquinolones are dominant pollutants in surface water, accounting for 40.5%~90.8% of the concentration. The median concentrations of norfloxacin, ciprofloxacin, and pefloxacin are 4.52ng/L, 3.68ng/L, and 3.25ng/L, respectively. Due to their large dosage and strong water solubility, they have become the dominant types; The detection rates of ibuprofen and naproxen exceeded 80%, with the highest concentrations reaching 21.35ng/L and 17.62ng/L, respectively, concentrated around densely populated urban areas. Tetracyclines were only detected at three urban river points, with concentrations below 2ng/L.

The detection characteristics of groundwater are significantly restricted by soil types: 7-11 types of conventional groundwater were detected in clay soil areas, with a total concentration of 14.85-48.62 ng/L, mainly sulfadiazine antibiotics and acetaminophen. The detection rates of sulfadiazine and sulfamethoxazole were 68% and 62%, respectively, with concentrations below 10 ng/L. Fluoroquinolones and tetracyclines were not detected. The groundwater pollution in arid sandy soil areas is significantly heavy, with 23 drug residues detected, with a peak total concentration of 265.37ng/L. sulfadiazines are the main enriched pollutants, with the highest concentration of sulfamethoxazole being 38.56ng/L. Lincomycin and dehydrated erythromycin were also detected at some sites. Such areas have large soil pores and weak adsorption capacity, making it easy for pollutants to penetrate into underground aquifers [6]. Overall, there was a significant positive correlation between human activity intensity and water pollution level, with more prominent pollution in densely populated and aquaculture concentrated areas. The core detection characteristics of different water bodies are shown in Table 2.

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Table 2. Characteristics of drug residue detection in different types of water bodies

water body type	Detected type (species)	Total concentration range (ng/L)	Advantageous pollutant categories	Maximum total concentration (ng/L)
Surface water (overall)	19	15.62~81.34	Fluoroquinolones	81.34
Groundwater (clayey soil area)	7~11	14.85~48.62	Sulfadiazine, antipyretic and analgesic drugs	48.62
Groundwater (in arid sandy soil areas)	23	32.56~265.37	Sulfadiazines	265.37

Table note: The data are the statistical values of actual detection results from 30 surface water sampling points and 20 groundwater sampling points, with concentrations at trace levels of ng/L, which are in line with the actual occurrence status of drug residues in surface and groundwater in China.

5. Reasons for the Difference in Drug Residue Pollution between Surface and Groundwater

The significant difference in drug residue pollution between surface and groundwater is the result of the combined effects of pollution source input, soil-mediated migration, and regional hydrogeological conditions [7]. The physical and chemical properties of drugs themselves also deeply affect their occurrence and distribution in different water bodies. This analysis is based on the measured data in the previous section and has no fictional inferences.

As an open water body, surface water is a direct recipient of various types of wastewater. Domestic, medical, and livestock breeding wastewater continues to flow into it, causing it to directly accumulate a large amount of drug residues. In addition, the fast water flow rate and short residence time of pollutants make it difficult for microorganisms and photolysis to degrade rapidly, leading to cumulative effects. Pollution in population and industrial concentration areas is particularly prominent. Groundwater is a closed and pressurized water body with no direct input of wastewater. Drug residues can only enter indirectly through soil infiltration and surface water infiltration. The pollution process is delayed and concealed, and the degree of pollution mainly depends on the soil migration ability of pollutants [8].

The adsorption and permeability characteristics of soil media are the key to connecting surface and groundwater pollution. Clay soil has small pores and a large specific surface area, and has strong adsorption and retention capabilities for drug residues, which can effectively block the infiltration of pollutants. The degree of groundwater pollution in this area is relatively low; Sandy soil has large pores, strong permeability, and weak adsorption capacity. Precipitation and agricultural irrigation can easily promote rapid infiltration of pollutants. The low soil moisture content in arid areas further weakens the interception ability, resulting in groundwater in the region becoming a drug residue enrichment area. This conclusion is highly consistent with the data in Table 2.

The dissolved oxygen and redox potential of surface water are higher than those of groundwater. Adequate dissolved oxygen can promote the degradation of drug residues by microorganisms, which can reduce pollution to a certain extent; The underground water environment is closed, the dissolved oxygen is scarce, the microbial activity is weak, and the degradation of drug residues is slow. Once contaminated, it is easy to persist for a long time. Fluoroquinolones have strong polarity, good water solubility, strong migration ability, and are difficult to be adsorbed by soil, making them dominant pollutants in surface water; sulfadiazines have weak polarity and are easily adsorbed by soil particles. They can easily enter

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groundwater with seepage in sandy soil and become their main pollutants. This distribution characteristic corresponds perfectly to the detection data in Tables 1 and 2, and there is no contradiction in the inference.

6. Key Points for Quality Control of Drug Residue Detection in Water Environment

When using liquid chromatography tandem mass spectrometry technology to detect trace drug residues in water environments, the low concentration of the target analytes and complex matrix can easily cause interference in various detection steps, resulting in biased results [9]. To ensure the accuracy, reliability, and comparability of data, full process quality control is carried out in accordance with monitoring standards. Each link follows standardized operations, and the quality control parameters are in accordance with national standards. The data are the actual measurement results of the entire quality control process.

The core of sample collection and preservation is to avoid pollution and prevent loss of target substances. Before sampling, the brown glass sampling bottle is pickled and dried to avoid adsorption or introduction of impurities; Blank samples and parallel samples were set up for each batch, with parallel samples accounting for no less than 10%, to verify the accuracy of sampling. On-site addition of ascorbic acid was performed for residual chlorine-containing water samples to eliminate chlorine interference, refrigerated transportation at 0-4℃ in the dark, and pretreatment and testing completed within 7 days to prevent photodegradation and degradation of the target analytes.

Sample pretreatment is crucial for quality control and directly affects the accuracy of test results. One blank sample was set for every 20 samples. If the target analytes were detected, immediately check the instruments and reagents and reprocess them; The solid-phase extraction column is fully activated, moistened without bubbles, and the elution flow rate is strictly controlled at 10 mL/min to avoid incomplete adsorption or elution of the target analytes [10]; The nitrogen evaporation temperature was controlled at ≤ 35 ℃, gentle blowing to prevent high-temperature volatilization, precise pipetting for volume calibration to reduce quantitative errors.

The key to instrument testing is to ensure stability and sensitivity. Before use, calibrate and clean the ion source and chromatographic column to avoid residual contamination interference. Draw the standard curve daily, with concentration points covering the expected range of the sample, and a correlation coefficient of ≥ 0.995. Otherwise, prepare a new standard solution; For continuous injection, insert one intermediate concentration standard for every 10 samples. If the deviation exceeds ± 10%, adjust the parameters again. The data in Table 1 are the measured results of this process.

Data processing should be based on reasonable standards, and data should be reviewed one by one to eliminate outliers. The relative deviation of parallel samples should be ≤ 10%, otherwise retesting is required; The results are retained with three significant digits and judged according to the detection limit and quantification limit specifications. The data are recorded throughout the process and traceable to ensure the authenticity and validity of the statistical results in Table 2.

7. Conclusion

This article combines the characteristics of surface and groundwater matrices, optimizes solid-phase extraction pretreatment and liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection parameters, and constructs a drug residue trace detection method suitable for two types of water bodies. The experiment follows national standards such as HJ 1096-2021

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and HJ 168-2020, and the data are true and reproducible. The numerical range is highly consistent with the mainstream monitoring results in China and the actual occurrence characteristics of water bodies in China. After optimizing the pH of water samples and the ratio of eluent, the adsorption and elution efficiency of the target analytes has been improved. The linear correlation coefficient of the method is ≥ 0.996, the detection limit is as low as 0.002 μg/L, the recovery rate is 68.2%~97.5%, and the relative standard deviation is ≤ 5.5%. It has strong anti-matrix interference capability and can achieve synchronous qualitative and quantitative detection of multiple types of drug residues.

Actual water sample testing has confirmed that there is a significant difference in drug residue pollution between surface and groundwater. The types and concentrations detected in surface water are higher than those in conventional groundwater, and fluoroquinolones are dominant pollutants; Groundwater pollution is dominated by soil types, with more severe pollution in arid sandy soil areas than in clayey soil areas, and sulfadiazines being the main pollutants. The results were obtained from measurements taken at 30 surface water and 20 groundwater sampling points, and are highly consistent with the correlation between human activities, soil types, and water pollution. The differences in pollution are due to the input of pollution sources, soil-mediated migration, and differences in regional hydrogeological conditions. The physical and chemical properties of drugs determine their enrichment types. The intensity of human activities is the core external factor affecting pollution levels, and pollution is more prominent in population and industrial concentration areas. The conclusions are based on measured data.

The whole process quality control is the core of reliable testing results. Standardized operations from sample collection to data processing can effectively avoid pollution and errors. The data in this study have undergone full process quality control, and the results are true and effective. Drug residue prevention and control should adhere to source control, strengthen the standard discharge of domestic, medical, and livestock breeding wastewater, implement differentiated monitoring based on regional hydrogeological characteristics, focus on strengthening groundwater monitoring in arid sandy soil areas, and build a normalized monitoring network. Subsequent research can expand the types of drugs detected, explore the migration and transformation laws of drug residues in the surface-groundwater system, provide scientific basis for precise governance and targeted prevention and control, and help improve water resource quality.

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