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Determination of sulfonamide antibiotics in sludge by ultrasonic solid phase extraction and HPLC

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Abstract: In order to improve the recovery rate of sulfonamide antibiotics in sludge, and to optimize the extraction and determination methods of sulfonamides antibiotics in sludge, ultrasonic solid phase extraction -HPLC was used for the detection of sulfonamides antibiotics in sludge. First the determination of the test factors by single factor test was conducted, then the extraction conditions were optimized by orthogonal experiment. When the extraction ratio of V (acetonitrile): V (water) was =1: 3, oscillation time was 20 min, ultrasonic frequency was 35 Hz, ultrasonic time was 15 min, and extraction agent was 20 ml, when the average content of extraction of sulfonamide antibiotics reached maximum, the recovery rates of Sulfadiazine, and sulfamethazine antibiotics were: 75.50% and 72.79% respectively. This indicates that the detection method can determine the content of sulfonamide antibiotics in the sludge effectively.

Introduction

In recent years, the environmental researchers pay more attention to the harmful and toxic effects antibiotic medicines in wastewater as a new type of pollutants in the environment.[1] Antibiotics are mainly used in the prevention and treatment of bacterial infections, and is one of the most widely used drugs in the world. Antibiotics have a potential impact on the development and spread of antimicrobial resistance. At present, the problem of antibiotic contamination has been listed as an important environmental problem in many developed countries in the first 20 years of twenty-first Century, and the lots of research has been going on to contain it [2]. It has been reported that in 2003, the output of sulfonamides antibiotics in China was more than 20000 t [3], which is widely used in clinical, animal agriculture and aquaculture [4]. Antibiotics, as human pharmaceuticals, are digested and excreted into the environment by human metabolites and are in unaltered form. At present, the antibiotic residues in drinking water [5], domestic sewage, soil, river sediment and sludge [6-7] and other environment has been detected. As the sewage treatment plant is considered to be the hub of various pollutants, the part of the drug is lipophilic that can enrich easily in sludge system, therefore, the detection of antibiotics concentration in the sludge can be as high as to be measured in mg/kg, which seriously is affecting the sludge compost, resource use, harmless disposal of sludge disposal and subsequent processing. In order to understand the existing level and pollution status of antibiotics in sludge, it is necessary to establish an analytical method for the determination of antibiotics in sludge, so as to test the actual samples.

Due to the complexity of the sludge matrix, there are many kinds of target drugs having different physical and chemical properties. In order to reduce the interference and improve the extraction efficiency, the samples extraction and purification steps are critical. Mingyue Li et al.[8] used matrix solid-phase dispersion and high performance liquid chromatography tandem mass spectrometry to analyze the typical antibiotics in sludge, and the recovery rate of sulfonamides was 40.15%-63.75%; Shuo Wang et al.[9] used ultrasonic extraction and high performance liquid chromatography tandem mass spectrometry to determine the antibiotic in sludge, and the recovery rate was 46.1%-69.6%; Xiaoxia Jiang [10] used ultrasonic extraction, HLB column pre-concentration and ultra-performance liquid chromatography mass spectrometry, with 10 ml 0.2 M citric acid buffer and 10 ml acetonitrile as an extraction agent, to determine the antibiotic in sludge sediment, and the recovery rate was 61.2%-96.3%; Jiali Ding et al.[11] used ultrasonic extraction, SAX-HLB column enrichment and HPLC tandem mass spectrometry, with methanol, 0.1 mol/L EDTA-2Na solution, and citric acid

buffer(pH=4) volume ratio of 3:1:2 as extraction agent, to determine the antibiotic in sludge sediments ,and the recovery rate was 57.4% -104.6%. In view of the research conducted so far, the acetonitrile and water were used as an extraction agent, the extraction conditions were optimized by orthogonal test, so as to improve the recovery rates of sulfadiazine and sulfamethazine.

Materials and methods

Instruments and reagents

Primary Instruments: ACQUITF high performance liquid chromatograph, QSE-12 Solid phase extraction apparatus (Shanghai Jingxin Corporation), Thermostatic oscillator, DN-12A Nitrogen blowing instrument (Wuxi Jiuping Instrument Corporation), analytical balance (Ohaus Corporation Corporation), Thermo high-speed centrifuge (Thermo Fisher Corporation), ultrasonic cleaner (Shanghai Branch ultrasonic instrument limited Corporation), freeze dryer, HLB Solid phase extraction column (6ml/200mg, Shanghai Amp Corporation), NH_2 purification column (6ml/200mg, Shanghai Amp Corporation).

Reagent: Methanol (HPLC grade, Merck Chemical Technology Corporation), acetonitrile (HPLC grade, Merck Chemical Technology Corporation), Acetone (HPLC, Sinopharm Chemical Reagent Co., Ltd.), Formic acid (purity 98%, Sinopharm Chemical Reagent Co., Ltd.), Ammonia (GR, Sinopharm Chemical Reagent Co., Ltd.), Hydrochloric acid (GR, Sinopharm Chemical Reagent Co., Ltd.), Ethylenediamine tetra-acetic acid disodium (EDTA-2Na, AR, Beijing Chemical Corporation), Deionized water is used in laboratory. Sulfadiazine (Beijing Bailingwei) purity 99%, Sulfamethazine (TCI Chemical Industrial Corporation) purity of more than 98%.

Standard solution preparation

Methanol was used as a solvent to prepare the standard storage solution with a mass concentration of 100 mg/L, which was stored at 4 ºC, and the standard solution was diluted with deionized water to prepare standard solution

Sample collection

The sludge for experiment was collected from the city of Shanghai Songjiang District sewage treatment plant sludge by plate filter press after (81.56% moisture content). Sludge samples collected were wrapped with aluminum foil and were immediately refrigerated the laboratory and stored at -20 ºC. After 12 hours freeze drying for 12 hours they were dry grinded in a mortar. Then the samples were 100 mesh sieved, after that they were mixed evenly and transferred to a closed glass bottle at 4 ºC for preservation.

HPLC conditions

Chromatographic column: Athena C_{18} column (4.6 x 150mm, 5um, Shanghai Amp Corporation), column temperature: 40 ºC, sample temperature: 4 ºC, sample volume: 20 μl, flow rate: 1 ml/min.

Mobile phase: A was methanol, B was 0.1% formic acid aqueous solution; mobile phase ratio: methanol was 30%, the aqueous solution of 0.1% formic acid was 70%.

UV detection wavelength: with reference to the relevant literature, sulfonamides detection wavelength at was 260-275 nm. A considerable part of the selection of the detectied wavelength was 265 nm [12]. Through the experiments of two sulfonamides standard samples in the 265 nm wavelength response was strong, sensitivity was high. Therefore, in this article the actual detection wavelength was at 265 nm.

Sample pretreatment

Sample extraction

1.00 gram of sludge was added to 50 ml centrifuge tube. Then added 10 ml of acetonitrile water (1:3) solution with ammonia and adjusted pH to about 10.0, the solution was shaken for 20 min at 35 Hz ultrasonic extraction 15 min, centrifugal 15 min (12000 r/min). After the remove of the supernatant and residue add 10 ml of extraction solution then the above operation was repeated twice. The extraction liquid was diluted with deionized water to 200 ml.

Sample purification and concentration

0.3 g of EDTA-2Na was added to the dilution liquid, pH value was adjusted with hydrochloric acid to 3.0. The dilution of all HLB cartridges (activation: 6 ml methanol, 6 ml 5% pH 3 EDTA-2Na aqueous solution), when all the diluted sample completion, the 10 ml of water was used to leach and vacuum. Then, 10 ml methanol was used to elute. The eluent flowed through NH_2 cartridge (activation: 6 ml acetone), then use 6 ml acetone - methanol - formic acid (500:500:1) solution and 6 ml acetone - formic acid (1000:1) solution was used for elution. The eluate was in soft under a stream of nitrogen blowing, blowing off to 1 ml, then used 10% methanol water solution to the sample bottle, volume 1ml, sealed refrigeration in 4 ºC to be saved, high performance liquid chromatography on the determination.

Results and analysis

Standard curve drawing

Standard dilution liquid reserves, with a standard solution of certain concentration. Using 50 μl micro syringe, volume of each sample was 20 μl, the concentration of 0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 5.0 mg/L, 10 mg/L, 100 mg/L of standard solution was determined by HPLC respectively. The standard curve was drawn using these values, the correlation coefficients were found to be greater than 0.99.

According to the retention time of sulfonamide antibiotics, compounds to be measured were identified. For the standard curves of 2 sulfonamide antibiotics (Table 1). The recovery rates for the two target antibiotics were 75.50%, 72.79% respectively, the correlation coefficient of two standard curves was 99.89%. Thus, this method possesses a good linear range and coefficient, and thus can satisfy the quantitative analysis needed for sulfonamide antibiotics.

Table 1 Retention time, linear relationship and recovery rate of two sulfonamides

Sulfonamides	Retention time（min）	Linear equation of standard curve	Correlation coefficient (R^2)	Recovery rate（%）
Sulfadiazine（SDZ）	2.959	y=1.5715x-0.0946	0.9989	75.50
Sulfamethazine（SDMD）	5.201	y=1.3172x-0.0555	0.9989	72.79

Single factor test

Study on the concentration of extraction agent

Acetonitrile in different concentrations (acetonitrile concentration were 15%, 25%, 50%, 75%) as the extraction solvent, ultrasonic and other operations under the 2.1 extraction methods, different concentration of extraction agent of sulfonamide antibiotics recovery effect is shown in Figure 1.

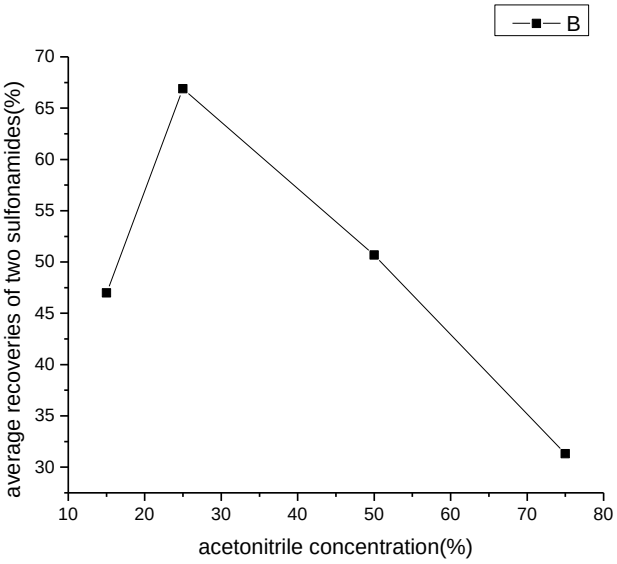

Figure 1 Effects of different acetonitrile concentration on the recovery of two sulfonamides

Figure 1 shows that different concentrations of acetonitrile as the extraction solvent, with the increase of the concentration of acetonitrile, the average extraction content of sulfonamide antibiotics firstly increases and then decreases. When the concentration of acetonitrile was 25% (acetonitrile: water =1:3), the extraction efficiency goes to the highest. Therefore the ideal range was found to be 25% of acetonitrile as an extraction solvent.

Investigation of oscillation time

A acetonitrile with a concentration of 25% was chosen as the solvent for the process. The time of oscillation was investigated at 10, 15, 20 and 25 min. The other operations were treated with 2.1 ultrasonic extraction methods. The effect of different shaking time on the recovery rate of sulfonamide antibiotics was shown in Figure 2.

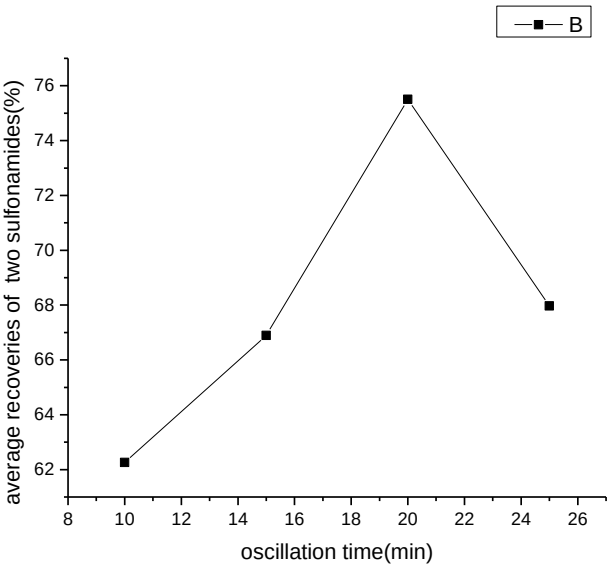

Figure 2 Effects of different oscillation time on the recovery of two sulfonamides

Figure 2 shows that with the increase of oscillation time, the average content of sulfonamide antibiotics showed a decreasing trend after the first increase, when the oscillation time was 20 min. The average content of sulfonamide antibiotics reaches to the highest point at, so 20 min therefore it is the ideal time for ultra-sonication.

Investigation of ultrasonic frequency

25% acetonitrile was chosen as an extraction solvent, the ultrasonic frequency of 35, 40 and 53 Hz was examined and the others operations were treated with ultrasonic extraction methods. The effects of different ultrasonic frequencies on the recovery rates of sulfonamides were compared with the results of Figure 3.

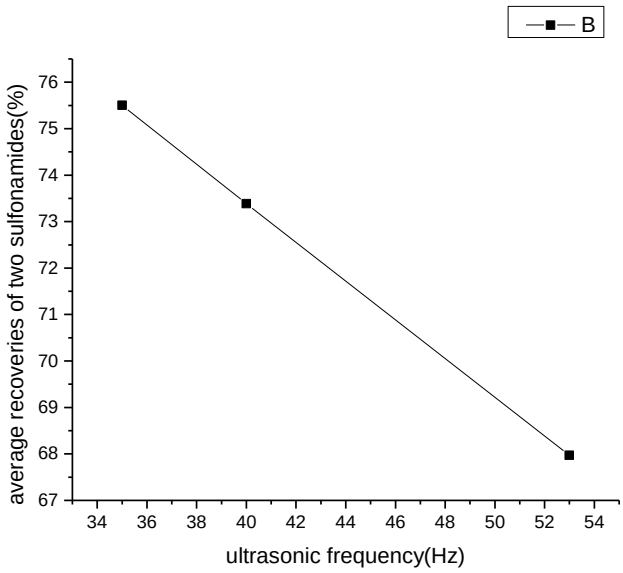

Figure 3 Effects of different ultrasonic frequency on the recovery of two sulfonamides

Figure 3 shows that with the increase of ultrasonic frequency, the average content of sulfonamide antibiotics showed a decreasing trend, when the ultrasonic frequency was 35 Hz, the average content of sulfonamide antibiotics reaches its highest, therefore 35 Hz is the ideal ultrasonic frequency.

Investigation of ultrasonic time

25% acetonitrile was used as an extraction solvent. The ultrasonic time was examined at 10, 15, 20 and 25 min, and the other operations were treated with 2.1 ultrasonic extraction methods. The effects of different ultrasonic time on the recovery rates of sulfonamide antibiotics were shown in Figure 4.

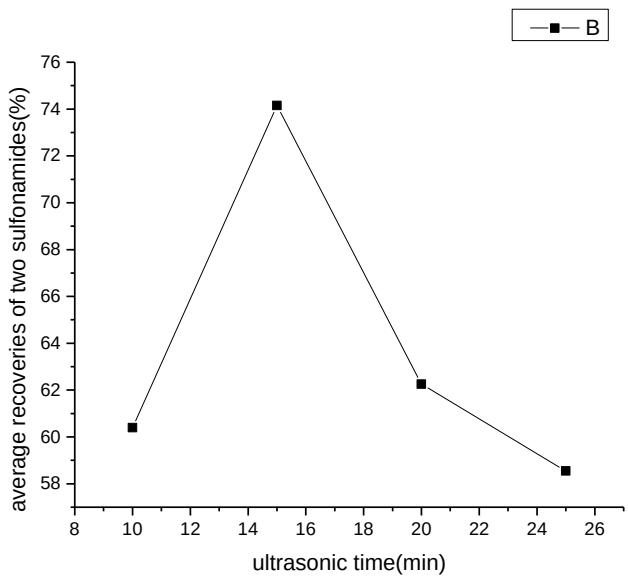

Figure 4 Effects of different ultrasonic time on the recovery of two sulfonamides

Figure 4 shows that with the increase of ultrasonic time, the average content of sulfonamide antibiotics showed a decreasing trend after the first increase, when the ultrasonic time was 15 min, the average content of sulfonamide antibiotics reaches the highest point, therefore 15 min is the ideal for ultra-sonication time.

Orthogonal test

Selection of extraction agent concentration (acetonitrile: water) (A), oscillation time (B), ultrasonic frequency (C) and ultrasonic time (D) are the four factors under consideration to conduct three level test. In which the extraction agent concentration were 1:1,1:3,3:1, the corresponding number of 1~3 (below), the oscillation time selection was for 10, 15 and 20 min, the ultrasonic frequency selection was 35, 40, 53 Hz, the ultrasonic time selection was 10, 15 and 20 min. In this orthogonal experiment, it can be seen that in $A_2B_3C_1D_2$ group (the extraction agent concentration was 1: 3, the oscillation time was 20 min, ultrasonic frequency was 35 Hz, ultrasonic time was 15 min), the average recovery rates of sulfonamide antibiotics were 74.15%, and the results were consistent with the previous single factor experiments.

Table 2 Orthogonal experiment table

Project	Influence factors				Average recoveries of two sulfonamides (%)
	A	B	C	D	
$A_1B_1C_1D_1$	1	1	1	1	46.31
$A_1B_2C_2D_2$	1	2	2	2	46.99
$A_1B_3C_3D_3$	1	3	3	3	50.68
$A_2B_1C_2D_3$	2	1	2	3	62.26
$A_2B_2C_3D_1$	2	2	3	1	66.89
$A_2B_3C_1D_2$	2	3	1	2	74.15
$A_3B_1C_3D_1$	3	1	3	1	31.32
$A_3B_2C_1D_3$	3	2	1	3	26.94
$A_3B_3C_2D_2$	3	3	2	2	27.68

In the data analysis of orthogonal experiment, the mean concentration of the extraction of three level tests were 47.993, 67.767 and 28.647 respectively, the range was 39.120; the mean shaking time of three levels were 46.630, 46.940 and 50.837, range was 4.207; the mean ultrasonic frequency of three levels were 49.133, 45.643 and 49.630, range was 3.987; the mean ultrasonic time of three levels were 48.173, 49.607 and 46.627, range was 2.980. From range comparison of four indicators, the changes in extraction agent concentrations had the most significant effect on the recovery rates of sulfonamide antibiotics, followed by the shock time, while the least impact was the ultrasonic time. Considering the four factors, take levels relative to the largest value for relative optimum namely $A_2B_3C_1D_2$. Under these conditions, the average recovery rate of sulfonamide antibiotics was 74.15% , and the relative standard deviation (RSD) was 9.085%.

Conclusions

(1)The method of ultrasonic solid phase extraction was used to optimize the extraction method of sulfonamide antibiotics and the aim of improving the recovery rate was achieved.

(2) To determine the factors of the orthogonal experiment by single factor test, namely the selection of extraction agent concentration (acetonitrile: water), oscillation time, ultrasonic frequency and ultrasonic time as four factors of orthogonal experiment. The extraction agent concentration of 1:3 was determined by orthogonal experiment, the oscillation time was 20 min, the ultrasonic frequency was 35 Hz, ultrasonic time was 15min. Under these conditions, the recoveries of 2 sulfonamides were 75.50% and 72.79% respectively.

(3) It is a specific target, which can be used for the determination of many kinds of sulfonamide antibiotics in sludge simultaneously, and can help to conduct the trace analysis of pollutants in sludge.

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